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Myocardial Injury in Critically Ill Patients with Co-existing Cardiovascular Disease

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Abstract

Approximately 30% of people admitted to ICU in the UK have co-existing cardiovascular disease (CVD), and this may rise as life-expectancy increases. Patients with CVD have impaired compensatory mechanisms to enable maximum oxygen delivery to the tissues in the event of critical illness, which itself increases global oxygen demand, further stressing the heart. This is exacerbated by tachycardia and hypotension, which may relatively reduce blood flow to the coronary arteries, and catecholamines which increase myocardial oxygen demand. The myocardium extracts 75% of the oxygen supplied by the coronary arteries at rest, and atheroma-related flow limitation further compromises myocardial oxygen delivery. However, the diagnosis of acute coronary syndrome in critical illness is not straightforward, due to patient inability to communicate symptoms, non-specific ECG changes, and poorly understood cardiac biomarker troponin elevation.

My overall hypothesis is that patients with CVD benefit from increased oxygen delivery to the myocardium during critical illness. A focus is the importance of anaemia. The aims of the studies presented in this thesis are (i) to systematically review the literature regarding blood transfusion thresholds specifically in patients with CVD; (ii) to explore the association between Troponin I (TnI) within 24 hours of ICU admission and hospital mortality (iii) to describe and quantify the dynamics of TnI in patients with CVD during the first ten days after ICU admission; and (iv) to define myocardial infarction in the context of critical illness.

I have performed a systematic review and meta-analysis of randomised controlled trials comparing a restrictive with liberal transfusion threshold and that included patients with CVD. In total, 11 trials enrolling patients with CVD (n=3033) were included for meta-analysis (restrictive n=1514, liberal=1519). The pooled risk ratio for the association between a restrictive transfusion threshold and 30 day mortality was 1.15 (95% CI 0.88 to 1.50, p=0.50, I^2=14%). The risk of acute coronary syndrome in patients managed with restrictive compared with liberal transfusion was increased (nine trials, risk ratio 1.78, 95% CI 1.18 to 2.70, p=0.0, I^2=0%). In contrast to broader literature supporting restrictive thresholds, our systematic review shows that a restrictive transfusion threshold of less than 80g/l may not be safe in patients with co-existing CVD, and highlights the variability in diagnostic definitions of ACS and the potential for ascertainment bias in transfusion trials.

I undertook a retrospective cohort study in two independently collected cohorts of general ICU patients who had TnI measured within 24 hours of ICU admission. Importantly, the majority of TnI samples were collected routinely rather than for clinical indications. We used the Abbott ARCHITECT Stat assay (limit of detection 0.01mcg/l. We performed multivariable regression, adjusting for components of the APACHE II model. We derived the risk prediction score from the multivariable model with TnI. TnI was associated with all cause hospital mortality (OR per doubling TnI 1.16, 95% CI 1.13 to 1.20, p<0.001) which persisted after adjustment for APACHE II model components (OR TnI 1.05, 95% CI 1.01 to 1.09, p=0.003). TnI correlated highly with the Acute Physiological Score component of APACHE II (r=0.39), suggesting that TnI release may be largely explained by acute physiological stress. Addition of TnI to the APACHE II model did not improve the performance of the risk prediction model and we would not advocate the adoption of a routine single troponin sample at admission.

I designed, set up, and recruited 279 patients to a prospective cohort study TROPonin I in Cardiovascular patients in CriticAL care (TROPICCAL, UKCRN 19253) in 11 UK centres. The aims were to (i) determine the incidence of Myocardial Injury and Infarction, defined by the Third Universal Definition of Myocardial Infarction; (ii) explore factors associated with Injury and Infarction from multivariable analyses; and (iii) explore the relationship between Injury/Infarction and outcome in unadjusted and adjusted analyses. We recorded baseline characteristics, and took daily hs-TnI for ten days after ICU admission, severity of illness measures and ECGs for 5 days.

There was a wide range of peak TnI (med 114ng/l (min 3, Q1 27, Q3 412, max 58820ng/l)) and a high prevalence of myocardial injury on systematic screening: 71% of patients had peak TnI greater than the sex-specific diagnostic threshold ("Injury"), and 24% had peak TnI greater than the sex-specific diagnostic threshold and dynamic changes on ECG consistent with ischaemia ("Infarction"). TnI consistently showed a rise-and-fall pattern consistent with an acute myocardial 'hit' rather than persisting injury, which peaked early during ICU stay. Importantly, only 12 (4.4%) patients were diagnosed with MI by the clinicians looking after the patients. Independent predictors of peak TnI in the preceding 24 hours were SOFA score, dynamic ECG ischaemia, lactate, haemoglobin, and age. The lack of association with CRP (representing systemic inflammation), with stronger association with lactate (representing inadequate perfusion/oxygen supply), Hb and ECG ischaemia support the conjecture that injury results in part from an acute ischaemic hit in this population. Patients with Infarction had similar baseline demographics to patients with Injury, but had higher peak TnI concentrations, and higher hospital and six month mortality (Figure 2). This supports the importance of including systematic assessment of dynamic ECG changes in the myocardial injury 'construct' in ICU.

My work has shown an increased risk of ACS in patients with CVD randomised to restrictive transfusion thresholds. TnI elevation is prevalent in general ICU patients, and is independently associated with hospital mortality. A systematic approach to the detection of myocardial injury in critically ill patients with co-existing CVD who are unable to communicate symptoms, can identify a high risk population who have poorer survival than patients with no injury. Markers of ischaemia are more associated with TnI rise than markers of inflammation, supporting the hypothesis that myocardial injury in this population is at least in part due to oxygen supply-demand imbalance "myocardial infarction".

From this work, I would recommend (i) a more liberal transfusion threshold of at least 80g/l in patients with co-existing CVD; (ii) systematic use of sequential ECGs in ICU to screen for myocardial injury in 'at risk' patients; and (iii) manipulation of physiological parameters such as anaemia, hypotension and tachycardia should be considered for patients with dynamic ECG changes plus troponin increase consistent with Infarction. Future research should include 'precision medicine' trials in the substantial cohort of ICU patients with co-existing CVD to explore whether interventions that increase myocardial oxygen supply and/or treat infarction alter outcomes.

Lay Summary

Approximately a quarter of patients admitted to Intensive Care, have co-existing cardiovascular disease (CVD). The heart muscle needs oxygen to function. A heart attack (myocardial infarction) occurs when there is death of the heart cells due to a lack of oxygen supply. The heart muscle is vulnerable in critical illness, and this is particularly true for patients with CVD who may already have narrowing of the coronary arteries that supply oxygen to the heart muscle. A heart attack is diagnosed by symptoms such as chest pain or breathlessness, levels of a protein called Troponin I (TnI) which is released during damage to the heart muscle, and signs on the heart tracing known as the electrocardiogram (ECG). It is difficult to diagnose a heart attack in patients in Intensive Care, as they are often confused, or sedated for medical treatment.

I performed a systematic review which showed that patients with co-existing CVD who were randomised to a lower blood concentration (and therefore potentially lower oxygen supply) had a greater risk of a heart attack than patients who were randomised to a higher blood concentration (and therefore potentially higher oxygen supply). This is different from patients without CVD, in whom the lower concentration has been shown to be at least as safe.

I collected data on critically ill patients with co-existing CVD looking at the levels of TnI and at daily ECGs. We found that two thirds of patients had a level of TnI higher than that required to diagnose damage to the heart muscle, and that a quarter of patients had TnI levels and ECG changes consistent with a heart attack. Only 4% of patients were diagnosed with a heart attack by the physicians looking after them. I found that variables that represented a lack of oxygen supply to the heart muscle (including a low blood concentration) were more closely associated with damage than variables that represented inflammation. This suggests that damage to the heart muscle in critically ill patients with co-existing CVD may at least be in part to an oxygen supply-demand imbalance. Patients who had a heart attack had lower survival up to six months than patients who had not.

From this work, I would recommend a maintaining a higher blood concentration in patients with co-existing CVD. I would also recommend systematic screening using ECGs and blood tests (TnI) to identify a high-risk group of patients who are frequently seen in our ICUs. This will enable us to direct treatment towards patients who may benefit the most, and avoid treating low risk patients. We have also identified interventions, such as blood concentration, for trials which may reduce the risk of heart attack in these patients.

Declaration

I declare that this thesis is of my own composition, and that the research contained within it is entirely my own unless stated otherwise. No part of the work has been submitted for any other degree or professional qualification. All included publications are my own work, and I am the first author.

Annemarie Docherty

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Relevant publications, presentations and awards

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AWARDS

SICS Travel Award May 2017, Scottish Intensive Care Society

NIAA Research Award Finalist 12 May 2017, National Institute of Academic Anaesthesia ASM. "Myocardial injury in critically ill patients with cardiovascular disease"

Maurice Hudson Prize, 14 December 2016, National Institute of Academic Anaesthesia (NIAA). "The impact of restrictive versus liberal transfusion strategies on patient outcomes in patients with cardiovascular disease excluding those undergoing cardiac surgery:

Nick Gordon Medal, 01 March 2016, Edinburgh and East Scotland Society of Anaesthetists (EESSA). "The impact of restrictive versus liberal transfusion strategies on patient outcomes in patients with cardiovascular disease excluding those undergoing cardiac surgery: A Systematic Review and Meta-analysis"

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1. Introduction

Haemoglobin (Hb) is the main transporter of oxygen to the tissues, and a fall in Hb concentration may result in inadequate oxygen delivery to vital organs with subsequent tissue hypoxia. Red blood cells are transfused to treat or prevent this inadequate oxygen delivery. However, in clinical practice we are unable to measure the intracellular oxygen tension and we are therefore forced to base our decisions to transfuse on surrogate measures.

This introductory chapter will explore the definition of anaemia, the limitations we face in determining adequate tissue oxygen delivery, and the concept of critical Hb concentration. I will discuss the epidemiology of anaemia in critical care, and its management. I will then focus on patients with cardiovascular disease and why anaemia is of particular importance in critically ill patients with cardiovascular disease. I will systematically review Hb thresholds for patients with co-existing cardiovascular disease (CVD) separately in Chapter 2. I will appraise the literature regarding myocardial injury in Chapters 3 and 4.

1.1. Definition of Anaemia

Aerobic metabolism allows cells to harness the energy stored in organic molecules more efficiently using oxygen as the final electron receptor(1). Anaemia from the Greek ἀναιμία, anaimia, meaning 'lack of blood', is defined by the World Health Organisation as a condition in which the number of red blood cells (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiologic needs (2). These physiologic needs vary with age, gender, pregnancy, smoking behaviour and certain environmental factors, such as altitude, and the WHO suggest minor variations in the thresholds to account for these factors. Current thresholds are haemoglobin <130g/l (haematocrit <39%) for adult males and <120g/l (haematocrit <36%) for adult non-pregnant females.

Symptoms of anaemia include fatigue, weakness, dizziness and reduced exercise tolerance. Acute and chronic anaemia have been associated with higher incidence of cardiovascular disease (3), cognitive impairment (4), acute kidney injury (5), decreased physical performance and quality of life, and increased risk of falls and fractures(6). Anaemia is also associated with increased mortality, increased length of hospital stay, and unplanned readmission rates (7, 8).

1.2. Overview of Oxygen transport

Global oxygen (O_2) delivery is mainly a product of cardiac output, haemoglobin concentration, and O_2 saturation. Haemoglobin is contained within erythrocytes, produced in the bone marrow of the long bones under the control of erythropoietin. Haemoglobin is a complex molecule consisting of four polypeptide globin chains, each incorporating an iron-containing heme ring where O_2 is bound according to its partial pressure. Each haemoglobin molecule can carry up to four oxygen molecules. Conformational changes lead to cooperativity among binding sites, meaning that once one oxygen molecule is bound, there is increased oxygen affinity of the other globin chains – this results in the characteristic sinusoidal oxygen-haemoglobin dissociation curve, where oxygen is bound at high oxygen partial pressures (the lungs), and released at low partial pressures (peripheral tissues) (9). This decrease in affinity is largely mediated by 2,3-diphosphoglycerate levels (2,3-DPG), but is also affected by temperature, pH and partial pressure of carbon dioxide (10). Oxygen is also dissolved in plasma,

however at 21% Oxygen concentration at Hb 140g/l this contributes only 2% to oxygen transport. As haemoglobin drops, this becomes progressively more important, and for a patient who is breathing 100% oxygen with Hb 50g/l, dissolved O_2 can account for 20% of O_2 transport (11).

1.3. Compensatory mechanisms in anaemia

As haemoglobin concentrations fall, compensatory mechanisms are activated, including increased cardiac output and increased oxygen extraction. Above Hb concentrations of 100g/l, compensation is mainly achieved by a rightward shift of the oxygen-haemoglobin dissociation curve due to increased 2,3-DPG, and by increased erythropoietin production which increases erythropoiesis (12). Below these concentrations, compensatory haemodynamic factors also occur:

In acute hypovolaemic anaemia, responses are mediated by the adrenergic and renin-angiotensin-aldosterone systems (13), primarily in response to hypovolaemia. Renin-angiotensin-aldosterone stimulation results in sodium and water retention. Sympathetic stimulation initially results in rises in heart rate and diastolic blood pressure, followed by further tachycardia, decreases in arterial pressure and diversion of blood towards coronary and cerebral circulations and away from splanchnic, cutaneous and skeletal circulations (13).

However, in chronic normovolaemic anaemia, increased cardiac output is mediated by lower afterload, increased preload and positive inotropic and chronotropic effects. Decreased afterload is a result of vasodilatation and reduced peripheral vascular resistance, which are in turn due to reduced blood viscosity, hypoxia-induced vasodilatation, and increased nitric oxide activity. Vasodilatation also causes increased capillary flow, recruitment of microvessels, and stimulation of angiogenesis which can limit the diffusion distance to cells. Preload increases due to increased venous return, which is also a result of reduced peripheral vascular resistance (10).

1.4. Measurement of oxygen delivery

We have no way to measure oxygen delivery directly. At present, arterial oxygen saturation and PaO_2 remain the principal clinical measures of arterial hypoxaemia. Mixed venous oxygen tension approximates to mean tissue oxygen tension and is useful to detect global decreases in tissue perfusion. However, a normal mixed venous oxygen tension does not rule out regional inadequate oxygen delivery, and in fact both low and elevated measurements are associated with a poor outcome in septic patients (14). Blood lactate is frequently used in combination with these as a surrogate measure of tissue hypoxia: inadequate cellular oxygenation results in anaerobic metabolism and production of lactic acid. Lactate has a poor sensitivity and specificity to detect microcirculatory alterations (15).

Oxygen consumption is a potential surrogate measure, as this falls when delivery can no longer meet metabolic demand. Shibutani et al estimated this critical value of oxygen delivery to be 8.2ml/min/kg (16), where below this oxygen consumption fell suggesting tissue hypoxia. Whilst potentially possible in a controlled research setting, there are too many uncontrolled factors that also impact on oxygen consumption in the clinical setting to be able to detect the fall due to inadequate delivery.

Gastric tonometry measures the partial pressure of CO_2 in the stomach. When the perfusion of gastric mucosa is reduced, CO_2 accumulates and this can be used in combination with systemic bicarbonate concentrations to

evaluate the adequacy of gastrointestinal mucosal perfusion. Trials have used this to guide inotropic/vasopressor therapy and red blood cell transfusion, but have not found significant improvement in mortality outcomes (17). The methodology of gastric tonometry has been questioned, as the systemic bicarbonate concentration may not reflect the concentration in ischaemic mucosa, and as a result, few institutions use gastric tonometry in clinical practice.

Near-infrared spectroscopy (NIRS) uses near-infrared light to compares the ratio of oxygenated to deoxygenated Hb, resulting in an indirect measure of tissue oxygenation (StO_2). It has been mainly used in cardiac surgery (18), and neonatal (19) and paediatric intensive care, and there is now increased interest in exploring its use in septic shock and neurocritical care (20). There is considerable intra- and inter-patient variability in the estimation of global oxygenation using NIRS. StO_2 values are mostly influenced by venous HbO_2 saturation, and this proportion varies between underlying conditions – in haemorrhage for example, the venous component is significantly reduced. NIRS is unable to detect perfusion heterogeneity, so that the interpretation of St O_2 is difficult as it does not represent capillary or mixed venous saturation. There is heterogeneity among studies assessing NIRS in the ICU (21). At present, NIRS is not used routinely in adult general ICUs.

1.5. Critical haemoglobin concentration

The critical haemoglobin concentration may be defined as the concentration at which compensatory mechanisms are exhausted, and further reductions in Hb will lead to a decrease in oxygen delivery, and subsequently oxygen consumption (22). Animal models have found this Hb around 40g/l (23, 24), although this depends on the metabolic activity and oxygen extraction capabilities of the tissues. Weiskopf et al performed acute isovolumetric reduction of Hb concentration to 50g/l in 32 conscious healthy resting humans. They found no evidence of inadequate oxygen delivery (using oxygen consumption and plasma lactate concentrations). Myocardial ischaemia occurred in only two patients, and resolved without sequelae (25). In a subsequent re-analysis of these data, he suggests that the mean Hb concentration associated with anaemia-induced mortality in humans is around 25g/l, and that cardiovascular disease increases that value (26).

Symptoms of anaemia such as breathlessness, fatigue, and angina, are related to the speed of onset of the anaemia. Patients with chronic normovolaemic anaemia, without cardiovascular comorbidity, may develop symptoms only when their haemoglobin concentration falls as low as 50g/l (13, 27). An analysis of Jehovah's Witness patients concluded that very few deaths due to anaemia occurred in patients with Hb>50g/l (28).

In a study of Jehovah's witnesses, Carson et al reported that for every 10g/l drop in postoperative haemoglobin below 80g/l, the odds of death increased 2.5 times and the odds increased 2.1 times for a composite outcome of 30-day mortality or morbidity (serious cardiac events, bacteraemia, pneumonia or deep wound infection) (29).

Hb concentration is measured because it is easy to quantify, and because it is responsible for a significant proportion of oxygen delivery. However, a simple concentration does not take into account the compensatory mechanisms mentioned above that occur in anaemia, and tells us nothing about global, or organ specific oxygen delivery. The critical Hb concentration will be different for each patient, and as we do not have the technology to reliably detect cellular hypoxia, we transfuse red blood cells in order to prevent its consequences.

Patients with disease pathologies such as cardiovascular disease may have a significantly higher critical haemoglobin concentration, and this will be discussed later in this chapter.

1.6. Anaemia in acute and critical illness

1.6.1. Prevalence

The prevalence of anaemia has been increasing in the past decade and is common in both acute and critical illness. The prevalence of anaemia in hospitalised patients is approximately 40-45% (6, 8, 30). Approximately two-thirds of patients admitted to ICU present with Hb concentration less than 120 g/l (31, 32), 39% present with Hb<100g/l (33), and 25% of patients present with Hb<90g/l (34). 97% become anaemic by Day 8, and in one study, 100% by day 13 (33). The prevalence of moderate to severe anaemia (Hb concentration <90g/l) at some time during ICU stay is approximately 40–50% among most ICU populations (35).

1.6.2. Pathophysiology

Acute haemorrhage is an important cause of anaemia, but there are many other causes of anaemia in acute and critical illness. These can be broadly divided into factors affecting red blood cell production, and factors affecting red blood cell survival.

Red cell production is suppressed in the presence of functional iron deficiency, reduced erythropoietin production and infection (32). Anaemia is usually normochromic and normocytic, and the erythropoietic response to anaemia is blunted(36, 37). This is similar to the anaemia seen in chronic inflammatory disease. Inflammatory cytokines including tumour necrosis factor alpha, interleukin-1 and interleukin-6 directly inhibit red cell formation (35). Inflammation also results in uptake of iron by macrophages, where it is incorporated into ferritin (38). This reduces the availability of free iron for erythropoiesis. Total body iron is likely to be normal, but this is difficult to assess as serum ferritin is increased and serum transferrin is decreased as part of the acute phase response (35). This is a functional iron deficiency where there is an inability to incorporate iron into haemoglobin despite normal iron body stores. Intestinal absorption of iron is inhibited by hepcidin, which is induced by pro-inflammatory cytokines (39). Hepcidin also inhibits the release of iron from tissue macrophages (40).

RBC survival may be shortened due to pathogen- and immune reaction-associated haemolysis (41). Haemodilution during resuscitation with colloid and crystalloid solutions will result in a reduction in Hb concentration, although red cell mass will remain constant. Frequent blood sampling alone may amount to 40-70ml per patient per day, accounting for 30% of required blood transfusions (42), and further losses may be due to occult gastrointestinal bleeding and haemolysis.

1.6.3. Anaemia after ICU discharge

There are little data regarding the prevalence and time course of anaemia in survivors of critical illness. One study showed that at hospital discharge 77% of patients remained anaemic, 33% had Hb<100g/l and 11.3% had Hb<90g/l. The strongest predictor of Hb<100g/l at hospital discharge was Hb at the time of ICU discharge (43). In a separate study, half of patients who were ventilated >24h and discharged from ICU with Hb<100, remained anaemic at six months (44). The anaemia was predominantly normochromic and normocytic, and was associated with ongoing inflammation, inappropriate erythropoietin response and poor marrow red cell production. Patients

had a markedly reduced health-related quality of life (HRQoL) at three and six months compared to the normal population. Compared with non-selected ICU patients, the mean scores were still reduced, particularly in the physical function and role physical categories. Outwith the critical care environment, studies have consistently shown an association between anaemia of chronic disease and HRQoL (45, 46). Fatigue is common in ICU survivors, and many of the physical features of post-ICU syndrome overlap with those of anaemia(47). However, the causal relationship has not been studied in this patient group.

1.6.4. Management of anaemia in critical illness

In the acute critical care environment, standard treatment for anaemia remains allogeneic red blood cell transfusion. Oxygen delivery is determined by cardiac output, Hb concentration and oxygen saturation. Increases in Hb concentration should therefore improve oxygen delivery to the tissues and to the myocardium itself and reduce the compensatory work done by the heart to increase cardiac output.

Other treatment options for anaemia include oral or intravenous iron, and erythropoietin. Free iron has been shown to potentiate bacterial growth(48), of particular concern in patients who are already critically ill. The uptake of oral iron is inhibited by hepcidin and, once absorbed, little of it is available for erythropoiesis. Furthermore, gastrointestinal side-effects such as constipation may limit its tolerance. Intravenous iron has been shown to be effective for increasing haemoglobin concentration outwith the ICU, but was associated with an increased risk of all cause infection (49). There have been two recent randomised controlled trials of intravenous iron in critically ill patients (50, 51). The IRONMAN study found that iv iron resulted in a higher Hb concentration at hospital discharge, but did not significantly reduce the number of RBC units transfused (51). Pieracci et al found that there was a significant increase in serum ferritin concentration, but no effect on Hb concentration or RBC transfusion requirement (50). Erythropoietin is essential for the production of RBCs in the bone marrow and is commonly used for patients with anaemia of chronic disease in cancer and chronic kidney disease. It suppresses apoptosis of erythroid colony-forming units, increasing numbers of normoblasts, and then reticulocytes after three to four days (52). Critically ill patients often display resistance due to inflammatory mediators requiring very high doses of ESAs up to ten times that used in chronic kidney disease and may only increase Hb concentration by up to 1.5g/l/day, compared with 10g/l after transfusion of one RBC unit (52). Furthermore, in critically ill patients no significant effect on overall mortality, duration of mechanical ventilation or ICU length of stay has been shown, and there are concerns regarding excess thrombotic events (53).

1.7. Red Blood Cell transfusion

1.7.1. Potential risks of transfusion

Anaemia is generally well tolerated by many hospital patients and therefore the benefits of red blood cell transfusion need to be weighed against the potential risks. The risk of transfusion-transmission infections, such as HIV, hepatitis B and hepatitis C, has reduced significantly over the past few decades and are now very low (USA, risk of HIV per unit of red blood cells 1:1,467,000 (54); hepatitis C 1:1,149,000 (54); hepatitis B 1:282,000 to 1:357,000 (55)). Other general risks include transfusion reactions, transfusion related acute lung injury (TRALI), and transfusion associated circulatory overload (TACO) (56, 57). The Serious Hazards Of Transfusion (SHOT) report on 2015 reported a risk of mortality of 1.01 per 100,000 components issued (total

deaths n=26, including haemolysis (n=5), TACO (n=7), and delayed transfusion (n=6)), and risk of major morbidity of 6.44 per 100,000 (TACO: n=34, Delayed transfusion: n=5, Acute transfusion reactions: n=86) (58).

RBC transfusion has been associated with immune modulation (TRIM). Potential mechanisms include suppression of cytotoxic cell and monocyte activity, release of immunosuppressive prostaglandins, inhibition of interleukin-2 production and increase in suppressor T-cell activity (59). Several studies have found an association between perioperative RBC transfusion and tumour recurrence (60). However most perioperative studies have been observational, and it is possible that the number of units transfused is due the complexity of the surgery and tumour size and invasiveness. The increased mortality seen in patients with large transfusions thus reflects the later stage of the tumour rather than an effect from transfusion. Immunosuppression has also raised concerns about potential infection, and meta-analyses have found that transfusion was associated with a significantly increased risk of postoperative infection(61-63).

It may also be that although the allogeneic red cells increase the oxygen content of the blood, the effects of storage on the red cells (the "storage lesion") render them significantly less effective and potentially harmful. The concentration of 2,3-DPG reduces rapidly, increasing the affinity of the red cells for oxygen and thus potentially reducing offloading of oxygen at the tissues (11). However, 2,3-DPG is regenerated in vivo, returning to near normal concentrations within a day of transfusion, and the clinical impact of this has not been proven. There is loss of production and increased consumption of red cell nitric oxide. Concentrations of adenosine triphosphate fall, resulting in morphological changes and decline in membrane lipid content and cell rigidity. The stiffened cells undergo haemolysis at a higher rate, leaching out free Hb and cytokines into the circulation, which then cause inflammatory and immunomodulatory responses and local vasoconstriction (64, 65). However, transfusion of fresh red cells, as compared with standard-issue red cells, has not improved survival among critically ill adults (66, 67). Red blood cell transfusion is expensive, each unit itself costs around £120 in the UK(68). This does not take into account the costs of acquiring the blood, or its safe administration, which may triple the costs (69).

1.7.2. Transfusion thresholds

Haemoglobin concentration is the parameter most commonly used to make decisions regarding RBC transfusion. However, there is uncertainty regarding the optimal transfusion threshold where benefits of additional oxygen carrying capacity outweigh risks of transfusion. Historically, patients were transfused if Hb concentrations fell below 100g/l (or haematocrit <30%) (70). 41 randomised controlled trials have since compared "restrictive" (patients are transfused when their Hb concentration falls below 70-80g/l) and "liberal" (patients are transfused at a higher Hb concentration of 90-100g/l) transfusion thresholds in hospitalised patients (Table 1). Two recent meta-analyses showed no increased risk of 30 day mortality or morbidity using a restrictive threshold compared with a more liberal threshold(64, 71) in general hospital patients. However, there were insufficient data for some subgroups including acute coronary syndrome and myocardial infarction. A review published in 2014 using restrictive thresholds of 70g/l showed reductions in mortality, rebleeding, acute coronary syndrome, pulmonary oedema and bacterial infections compared with liberal thresholds (72).

Table 1: Characteristics of blood transfusion trials comparing restrictive with liberal transfusion thresholds. S: Single centre, M: Multi centre, R: restrictive, L: liberal

Author	Journal	Clinical Setting	Thresholds	Total n	Primary endpoint
Almeida 2013 Brazil (S)	Critical Care	Oncology	R: 70g/l	101	Composite death or severe complications
			L: 90g/l	97	
Bergamin 2014 Brazil (S)	Critical Care	Critical Care	R: 70g/l	73	28 day mortality
			L: 90g/l	63	
Blair 1986 UK (S)	British Journal of Surgery	GI haemorrhage	R: 80g/l or shock	26	Clotting, death
			L: 2 units	24	
Bush 1997 USA (S)	American Journal of Surgery	Elective Vascular surgery	R: 90g/l	50	Myocardial ischaemia, myocardial infarction, death
			L: 100g/l	49	
Carson 1998 USA/Scotland (M)	Transfusion	Hip fracture patients	R: 80g/l or symptoms of anaemia	42	60 day mortality
			L: 100g/l	42	
Carson 2013 USA (M)	NEJM	Hip fracture patients with CVD/risk factors for CVD	R: 80g/l or symptoms	1009	60 day mortality Walk unaided
			L: 100g/l	1007	
Carson 2013 USA (M)	American Heart Journal	Symptomatic Coronary Artery Disease	R: 80g/l	55	Composite: all cause mortality, myocardial infarction or unscheduled coronary revascularization
			L: 100g/l	55	
Colomo 2008 Spain (S)	Hepatology (abstract)	GI haemorrhage in cirrhosis	R: 70g/l	109	mortality
			L: 90g/l	105	
Cooper 2011 USA (M)	American Journal of Cardiology	Acute MI	R: hct <24%	24	Composite: In-hospital death, recurrent MI, new or worsening congestive heart failure
			L hct<30%	21	
Fan 2014 China (S)	Archives of Gerontology and Geriatrics	elective Total hip replacement	R: 80g/l or symptoms of anaemia	41	Postoperative Delirium (POD)
			L: 100g/l	42	
Fortune 1987 USA (S)	Journal of Trauma	Traumatic patients with class 3-4 haemorrhagic shock	R: hct near 30%	12	Metabolic stability
			L: hct near 40%	13	
Foss 2009 Denmark (S)	Transfusion	Hip fracture patients	R: 80g/l	60	Postoperative functional mobility
			L: 100g/l	60	
Gregersen 2015 Denmark (S)	Acta Orthop	Frail elderly hip fracture patients	R: 97g/l	116	Recovery from physical disabilities
			L: 113g/l	111	
Grover 2006 UK (S)	Vox Sanguinis	orthopaedic surgery	R: 80g/l	109	Silent myocardial ischaemia
			L: 100g/l	109	
Haberkern 1997 USA (M)	Blood	sickle cell surgery	R: conservative	110	Sickle cell events
			L: aggressive	120	
			Not randomised	134	
Hebert 1995 Canada (M)	JAMA	Critical care	R: 70g/l	33	Feasibility Mortality
			L: 90g/l	36	
Hebert 1998	NEJM	Critical care	R: 70g/l	418	30 day mortality

Canada (M)			L: 90g/l	420	
Hochain 1996	Gut (abstract)	variceal bleeding	R: PCV 25	43	Rebleeding
			L: PCV 32	47	
Holst 2014 Scandinavia (M)	NEJM	Critical Care	R: 70g/l	502	90 day mortality
			L: 90g/l	496	
			L: 120g/l		
Jairath 2015 UK (M)	Lancet	Upper GI haemorrhage	R: 80g/l	403	Feasibility
			L: 100g/l	533	
Koshy 1988 USA (S)	NEJM	sickle cell pregnancy	R:as required	36	Perinatal mortality
			Prophylactic transfusion	36	
Liu 2015 China (S)	Chinese Medical Journal	Emergency surgery	R: illness severity score	33	Feasibility
			L: standard care	32	
Lotke 1999 USA (S)	Journal of Arthroplasty	Total knee arthroplasty	R: 90g/l	62	Not explicit Hb, LOS, wellbeing
			L: 2 units starting in recovery room	65	
Mazza 2015 Brazil (M)		Critical Care	R: 70g/l	22	Effect of transfusion on lactate and SvO_2
			L: 90g/l	24	
Nielsen 2012	Transfusion medicine	Spinal surgery	R: 73g/l	25	subcutaneous oxygen tension
			L: 89g/l	23	
Nielsen 2014 Denmark (S)	BMC Anesthesiology	Elective hip revision	R: 73g/l	33	Timed Up and Go-test
			L: 89g/l	33	
Park 2008 South Korea (S)	Cancer Chemo and Pharm	Gastric oncology	R: 100g/l		
			L: 120g/l		
Parker 2013 UK (S)	Injury	Hip fracture patients	R: definite symptoms of anaemia	100	30 day mortality
			L: raise Hb to >100g/l	100	
Palmer 1998 Scotland (S)	Transfusion Medicine (abstract)	hip fracture			
Prick 2013 Netherlands (M)	BJOG	postpartum haemorrhage Hb 48-79g/l	R: no transfusion	262	Physical fatigue
			L: transfusion	259	
Robertson 2014 USA (M)	JAMA	Traumatic brain injury	R: 70g/l	99	Glasgow Outcome Scale
			L: 100g/l	101	
So-Osman 2004 Netherlands (S)	Vox Sanguinis (abstract)	hip/knee surgery	Unclear		Blood use
So-Osman 2010 Netherlands (M)	Vox Sanguinis	Elective Total knee or hip replacement	R: stratified by risk	299	Hospital LOS
			L: standard care	304	
Villanueva 2013 Spain (S)	NEJM	Upper GI bleeding	R:70g/l	444	Mortality at 45days
			L: 90g/l	445	
Villarejo 1999 Spain (S)	Acta Gastroenterol Latinoam.	GI haemorrhage	R: hct 21%		
			L: hct 28%		
Walsh 2013 UK (M)	Critical Care Medicine	Critical care	R: 70g/l	51	30 day mortality
			L: 90g/l	49	
Webert 2008 Canada (M)	Transfusion	Leukaemia	R: 80g/l	29	Bleeding
			L: 120g/l	31	

Weiss 1982 USA (S)	Lancet (letter)	Acute leukaemia	R: as needed	12	Marrow recovery
			L: 120g/l	12	
Wu 2011 China (S)	Intensive Care Medicine (abstract only)	liver transplant	R: 70g/l	112	30 day mortality
			L: 100g/l	114	
Zheng 2013 China (S)	Experimental and Therapeutic Medicine	Orthopaedic surgery elderly patients	Standard	52	blood transfusion effectiveness
			Goal directed	54	
Zygun 2009 UK (S)	Critical Care Medicine	traumatic brain injury	R: 8g/dl		Change in brain tissue oxygenation
			Mid: 9g/dl		
			L: 10g/dl		

1.7.3. Guidelines for transfusion

The majority of guidelines now recommend a restrictive haemoglobin threshold for haemodynamically stable patients without major comorbidity (Table 2). However, they acknowledge the uncertainty in the evidence in the presence of severe comorbidity.

Table 2: Guidelines for transfusion thresholds

Organisation	Year	Recommendation	Recommendation for CVD
American Society of Anesthesiologists (73)	2006	60g/l	60-100g/l dependent on comorbidity and organ ischaemia
The American College of Critical Care Medicine	2009	70g/l	Higher for acute myocardial infarction or unstable myocardial ischaemia
American Association of Blood Banks (74)	2012	70g/l	Transfuse patients with symptoms of Hb<80g/l
British Committee for Standards in Haematology (75)	2012	70g/l, target 70-90g/l	Stable angina should have Hb>70g/l
National Institute for Health and Clinical Excellence (NICE) (76)	2015	70g/l, target 70-90g/l	ACS: transfusion threshold of 80g/l, target of 80-100g/l. Chronic: further research
Association of Anaesthetists of Great Britain and Ireland (AAGBI) (77)	2016	70g/l	Uncertainty remains for patients with IHD, higher thresholds (80g/l) may be appropriate

1.8. Patients with Cardiovascular Disease

Cardiovascular disease (CVD) is the collective term for all diseases affecting the heart and blood vessels. It is defined for the purposes of this thesis in Table 3. Statistics from the British Heart Foundation show that approximately seven million people in the UK have co-existing CVD(78, 79). In 2014, CVD was the second commonest cause of death in the UK, accounting for 27% of all deaths, 25% of premature deaths in men, and 17% of premature deaths in women. There is considerable geographical variation in mortality rates: in 2014 the rates were highest in Scotland and the North of England, and lowest in the South of England. The death rate from coronary heart disease in Scotland is 45% higher than the South East of England, and the premature death rate is 72% higher. CVD accounted for 10% of all NHS inpatient episodes in men, and 6.2% in women. The economic burden of CVD is high: in 2013/14 there were 1.7 million hospital admissions for CVD, NHS England spent £4.3 billion on treating CVD, Wales spent more than £430 million, and in 2011/12 Scotland spent nearly £800 million (79).

Table 3: Definition of Cardiovascular Disease

Cardiovascular Disease	The collective term for all diseases affecting the heart and blood vessels
Acute Coronary Syndrome	Acute Myocardial Infarction (MI) Anginal symptoms Electrocardiographic changes consistent with ischaemia Biomarker elevation
Chronic Cardiac Disease	Ischaemic Heart Disease Left/Right/Congestive Cardiac Failure Valvular disease (non-endocarditis associated) Chronic arrhythmia on treatment Hypertensive heart disease Hypercholesterolaemic/hyperlipidaemic heart disease
Cerebrovascular Disease	Cerebrovascular Accident (CVA) Transient Ischaemic Attack (TIA)
Peripheral Vascular Disease	Abdominal Aortic Aneurysm (AAA) +/- Thoraco- (TAAA) Vascular surgery Symptoms of vascular insufficiency under vascular review
Age >75 with Diabetes or Hypertension	Type I or II Diabetes on medication Hypertension on medication

1.8.1. Myocardial Infarction

Myocardial infarction (MI) is defined as evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischaemia. It can be recognised by the presence of symptoms, electrocardiographic (ECG) evidence of myocardial ischaemia, a rise/fall pattern of biomarkers of myocardial necrosis (usually Troponin I or Troponin T), and by imaging, or may be defined by pathology (80).

1.8.2. Myocardial Injury in Critically ill patients

Diagnosis of myocardial infarction is potentially more difficult in critically ill patients. Patients may be unable to communicate their symptoms, either due to therapeutic sedation, ventilation and analgesia, due to delirium, or other significant distracting injuries. ECGs are not performed routinely, and there is significant inter-observer variability in critical illness (81). Troponin release is prevalent in the critically ill (82), and there are multiple causes. Potential cardiac causes include Type I MI (increased thrombogenicity leading to coronary artery plaque rupture and thrombosis), and Type II MI (myocardial oxygen supply-demand imbalance). There are also non-coronary artery causes including sepsis, end stage renal disease, pulmonary disease and acute intracerebral pathology (83). Imaging is presently mainly limited to transthoracic echocardiography (TTE) as transfer of unstable patients to isolated locations such as the CT scanner, or angiography carries considerable risk. TTE is technically challenging in ventilated patients, and has only moderate diagnostic accuracy (84).

1.8.3. Anaemia in Cardiovascular Disease

The myocardium has a limited anaerobic capacity, and is dependent on a continuous supply of oxygen from the coronary circulation. At rest, the coronary blood flow is approximately 250ml/min, representing 5% of cardiac output. The myocardium extracts 75% of the oxygen, which cannot increase significantly in response to increased oxygen demand, and the coronary sinus PO_2 is subsequently very low (3kPa) (85). In order to match the considerable increases in myocardial O_2 consumption during exercise (up to five times resting consumption), there must be substantial increases in coronary blood flow. Flow across the myocardium largely depends on the pressure gradient between the aortic root and the right atrium. The force from the contracting heart muscle is greatest in the left ventricular subendocardial layers where it approximates to intramyocardial pressure, and significant left ventricular coronary flow can occur only during diastole. The right coronary flow is less affected by systole because of the smaller right ventricular muscle mass. It follows that any increase in heart rate will result in a reduction in diastolic time and will reduce perfusion time. Blood flow is also controlled by the diameter of the coronary arteries. This is under nervous and humoral control as well as local vasorestrictors and vasocontractors in the endothelium. Hypoxia causes coronary vasodilatation directly, and also releases adenosine and opens ATP-sensitive potassium channels (86).

Anaemia is associated with worse outcomes in patients with CVD, both in terms of severity of illness and mortality. Anaemia is associated with poor outcome in ischaemic heart disease (87), chronic heart failure (88), rhythm disturbance, and mortality and major adverse cardiovascular events in acute coronary syndrome(3). This may be due to increased myocardial workload and adverse left ventricular and large artery remodelling. Anaemia is associated with worse post-operative mortality in patients with CVD (89), suggesting that patients with co-existing CVD are less tolerant of anaemia than patients without CVD. This is consistent with animal studies which showed that dogs with experimentally created coronary stenoses developed ischaemic ECG changes at higher Hb concentrations compared with those with normal coronary arteries (stenoses: 70-100g/l vs normal: 30-50g/l) (90). However, the evidence is mainly from observational studies, and it is difficult to tease out whether the anaemia is exacerbating the underlying condition, or is a reflection of the severity of the underlying disease. It follows therefore, that reversing anaemia with RBC transfusion may not improve patient prognosis.

1.8.4. Anaemia in critically ill patients with CVD

Anaemia causes an increase in cardiac output, achieved by an increase in heart rate and stroke volume. In acute and critical illness, the presence of hypotension and tachycardia reduce blood flow through the coronary arteries, and the use of catecholamines increase myocardial O_2 demand. Global O_2 demand is also increased. The myocardial oxygen supply is reduced in patients with anaemia, and patients with co-existing cardiovascular disease, with potentially atheroma related flow-limiting disease, have limited ability to compensate.

1.9. Conclusion

There is biological plausibility that patients with CVD may benefit from higher transfusion thresholds than patients without CVD. Important end-points include mortality, and MI. MI is difficult to diagnose in critically ill patients. Non-coronary myocardial injury is likely to be the main cause of cardiac biomarker release in patients without CVD, and there is not therefore a clear argument that increasing oxygen supply (by increasing

haemoglobin concentrations) would reduce myocardial injury. However critically ill patients with CVD are at high risk of oxygen supply-demand myocardial injury. At present, there is little understanding of the prevalence of myocardial injury in these patients, or the dynamics of cardiac biomarkers.

2. Hypothesis

Patients with cardiovascular disease benefit from higher oxygen delivery, principally through higher red blood cell concentrations, to the myocardium during critical illness.

2.1. Aims and Objectives

The principal aims of this thesis are to understand current strategies for red blood cell transfusion in critically ill patients with cardiovascular disease, and to explore the incidence of myocardial injury in this population. My aim in presenting this work in this thesis is to systematically summarise the current evidence in this area, and to undertake a number of cohort studies to better understand the prevalence and association of anaemia, myocardial injury, and mortality in critically ill patients with cardiovascular disease.

2.2. Objectives

The thesis will address these specific objectives:

1. To systematically review the literature relating to blood transfusion thresholds in adult patients with co-existing cardiovascular disease
2. To evaluate the association between Troponin I taken within 24 hours of ICU admission and hospital mortality in general ICU patients
3. To describe the incidence and pattern of anaemia in patients with CVD in ICU in the UK
4. To describe myocardial injury in critically ill patients with CVD
5. To discuss the design of a blood transfusion threshold trial for critically ill patients with CVD

2.3. Thesis structure:

I present a systematic review, published in the BMJ, of the current evidence for blood transfusion thresholds in adult patients with co-existing CVD (Chapter 3).

This is followed by a data-linkage project, published in Critical Care, looking at the association between TnI taken within 24 hours of ICU admission, and hospital mortality in general ICU patients (Chapter 4).

Chapter 5 describes the prospective cohort study TROPonin I in Cardiovascular patients in CriticAL care (TROPICCAL), which explores the dynamics and associations of TnI in critically ill patients with CVD.

In Chapter 6, I combine chapters 3 and 4, and look at the association between TnI taken within 24 hours of ICU admission, and hospital mortality in ICU patients with CVD.

In Chapter 7, I discuss a potential outline for a blood transfusion trial in critically ill patients with CVD.

In the final chapter, I discuss the overall strengths and weaknesses of the thesis, and integrate the findings from the chapters.

3. The impact of restrictive versus liberal transfusion strategies on patient outcomes in patients with cardiovascular disease excluding those undergoing cardiac surgery: A Systematic Review and Meta-analysis

There have been several high quality systematic reviews looking at blood transfusion strategies over the past few years. However, none of these have specifically addressed patients with cardiovascular disease, who may have a different risk-benefit balance for transfusion.

The following chapter was published in the BMJ in March 2016 (91), and has been included in its published format as per Edinburgh University guidelines.

The impact of restrictive versus liberal transfusion strategies on patient outcomes in patients with cardiovascular disease excluding those undergoing cardiac surgery: A Systematic Review and Meta-analysis

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Key Words: Red cell transfusion, cardiovascular disease, randomised controlled trial, systematic review, meta-analysis

Word count: 4,377

ABSTRACT

Objectives: To compare patient outcomes with restrictive vs liberal red cell transfusion strategies in patients with cardiovascular disease, excluding those undergoing cardiac surgery.

Design: Systematic review with meta-analyses of randomised controlled trials.

Data Sources: In-hospital red cell transfusion threshold randomised controlled trials. We searched (to 02/11/2015), CENTRAL; MEDLINE; Embase; CINAHL; PUBMED; LILACS; NHSBT Transfusion Evidence Library; ClinicalTrials.gov; WHO International Clinical Trials Registry Platform; ISRCTN Register; EU Clinical Trials Register. We contacted authors for relevant data whenever possible.

Trial selection: Published and unpublished randomised controlled trials that evaluated a restrictive vs liberal transfusion threshold and that included patients with cardiovascular disease.

Data extraction and synthesis: Data extraction was completed in duplicate. Risk of bias was assessed using Cochrane methodology. Relative risk ratios with 95% CI were presented in all meta-analyses. Mantel-Haenzel random effects models were used to pool the risk ratios.

Main outcome measures: Thirty-day mortality, and cardiovascular events

Results: We identified 41 trials; of these 7 trials included data on patients with cardiovascular disease. Data from a further 4 trials enrolling patients with cardiovascular disease was obtained from the authors. In total, 11 trials enrolling patients with cardiovascular disease (n=3,033) were included for meta-analysis (restrictive transfusion threshold, n=1,514 patients; liberal transfusion threshold, n= 1,519). The pooled risk ratio for the association between transfusion thresholds and 30 day mortality was 1.15 (95% CI 0.88 to 1.50, P=0.50) with little heterogeneity (I^2=14%). There was an increased risk of acute coronary syndrome (ACS) in patients managed with a restrictive compared with a liberal transfusion threshold (9 trials; RR 1.78, 95% CI 1.18 to 2.70, P=0.006, I^2= 0%).

Conclusions: The results show that it may not be safe to use a restrictive transfusion trigger below 80g/l in patients with ongoing ACS or chronic cardiovascular disease. Effects on mortality and other outcomes are uncertain. These data support the use of a more liberal transfusion threshold (greater than 80g/l) for patients with both acute and chronic cardiovascular disease until adequately powered high quality randomised trials have been undertaken in patients with cardiovascular disease.

Trial registration: PROSPERO: CRD42014014251(1); http://www.crd.york.ac.uk/PROSPERO/).

Word count: 342

"What this paper adds"

"What is already known on this subject"

Restrictive red cell transfusion policies are recommended as safe for the majority of hospital patients with anaemia.

Uncertainty exists for patients with cardiovascular disease, in whom the heart may be more susceptible to limited coronary oxygen supply.

No previous systematic reviews have specifically compared outcomes for patients with cardiovascular disease outwith the cardiac surgery setting, and guidelines acknowledge the paucity of evidence in this area.

"What this study adds"

This review indicates that restrictive blood transfusion strategies may not be as safe as more liberal transfusion strategies for patients with co-existing cardiovascular disease in non-cardiac surgery settings.

Specifically, we have shown an increased risk of acute coronary syndrome with restrictive triggers below 80g/l.

These data support the use of a more liberal transfusion threshold (greater than 80g/l) for patients with both acute and chronic cardiovascular disease, until adequately powered high quality randomised trials have been undertaken in this patient population.

INTRODUCTION

Approximately 7 million people in the UK have cardiovascular disease (78), and it is a prevalent comorbidity among hospitalised patients. In observational studies anaemia is associated with worse outcomes in patients who have both acute and chronic cardiovascular disease, but it is unclear whether this association is causal or whether correction with red cell transfusions modifies this relationship(32, 89, 92, 93). Anaemia both decreases the oxygen content of the blood supplied to the myocardium and may increase myocardial oxygen demand because a higher cardiac output is required to maintain adequate systemic oxygen delivery (3). The heart extracts a high proportion of the oxygen supplied via the coronary arteries, and therefore this circulation is potentially at higher risk from the combination of atheroma-related flow limitation and anaemia. Hypotension, tachycardia and the requirement for catecholamine use, for example during critical illness or major surgery, can further compromise oxygen supply-demand balance resulting in myocardial injury. This has been termed type 2 myocardial infarction (94). Troponin release, a biomarker of myocardial injury, is associated with higher mortality in critically ill and perioperative populations (95-97).

Systematic reviews of randomised trials of liberal versus restrictive blood transfusion strategies support a general default trigger of around 70g/l for most patient groups (71, 98, 99), and this is reflected in recent guidelines advocating restrictive use of blood transfusions (74, 75, 77, 100). These have highlighted the lack of evidence and uncertainty regarding best practice for patients with acute or chronic cardiovascular disease (74, 75, 77, 100). No systematic reviews have specifically compared outcomes for patients with chronic cardiovascular disease undergoing non-cardiac surgery or other treatments such as intensive care. A recent systematic review restricted to patients undergoing cardiac surgery suggested better outcomes with more liberal transfusions, highlighting the potentially important interaction between anaemia, blood transfusions and outcomes for patients with cardiovascular disease (101). The National Institute for Health and Care Excellence (NICE) blood transfusion guideline, published in November 2015, stated that the optimal transfusion threshold for patients with ongoing acute coronary syndrome was 80-100g/l, but made no specific recommendation for patients with chronic cardiovascular disease and highlighted the need for further research in this specific population (76).

We conducted a systematic review and meta-analysis, for the first time, assessing the effect of restrictive vs liberal red cell transfusion strategies on patient outcomes restricted to adult patients with cardiovascular disease excluding patients undergoing cardiac surgery.

METHODS

This systematic review was conducted according to the protocol registered with PROSPERO (registration no: CRD42014014251(102); http://www.crd.york.ac.uk/PROSPERO/). We followed methods defined in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines statement(103).

Eligibility criteria

We included only randomised controlled trials (RCTs). RCTs were eligible for inclusion if they evaluated the effectiveness of any policy involving the use of a trigger or transfusion threshold based on haemoglobin concentration (including haematocrit) for guiding allogeneic red cell transfusion. Control group patients were required to be transfused at a higher haemoglobin concentration or haematocrit. We considered trials including adult patients (≥18yrs) except those involving cardiac surgery because this is a distinct group of patients, whose cardiovascular risk has been significantly altered by their procedure (102) (Figure 1). We excluded children and neonates due to the low prevalence of cardiovascular disease.

We defined Cardiovascular disease in our protocol as: known Coronary Artery Disease (CAD): Acute Coronary Syndrome (ACS), chronic Ischaemic Heart Disease (IHD); or other Cardiovascular Disease - Cerebrovascular Accident (CVA), Transient Ischaemic Attack (TIA), Peripheral Vascular Disease (PVD). We defined Acute Coronary Syndrome as: ST elevation myocardial infarction, non-ST elevation myocardial infarction, or unstable angina. A summary of the definitions for cardiovascular disease used by authors of included trials is presented in Table E1.

Search strategy

We did not restrict our search by language, date or publication status. We updated a search strategy we conducted in September 2009 reviewing the overall use of red blood cell (RBC) transfusions(104)(22). The present search included two changes: a) in CENTRAL there was a date restriction and b) the following search method was used in MEDLINE and Embase: i) the original search strategies + original RCT filters were re-run up until the end of 2008; ii) the new strategies + new RCT filters were run for all years; iii) the results of i) were then removed from the new search results. Once all the search results had been downloaded into bibliographic software, all previously screened references from the overview of the use of RBC transfusion, along with any duplicates, were removed. Search strategies are available in the online data supplement.

The date of the last search was 2nd November 2015 for the following databases:

CENTRAL (The Cochrane Library Issue 8, 2014): publication years from 2009-2014; MEDLINE (1946 onwards); Embase (1974 onwards); CINAHL (1937 onwards); PUBMED (epublications only); LILACS (2009-2014); TRANSFUSION EVIDENCE LIBRARY (1980 onwards); Web of Science (Conference Proceedings Citation Index- Science (CPCI-S) -1990 to present).

Ongoing Studies were searched for on five registries: ClinicalTrials.gov; WHO International Clinical Trials Registry Platform (ICTRP); ISRCTN Register; European Union Clinical Trials Register (https://www.clinicaltrialsregister.eu/ctr-search) and the Hong Kong Clinical Trials Registry. All sites were searched on 2nd November 2015. For detailed information regarding search strategies see supplementary appendix 1.

Data extraction

Trial selection

Two authors (AD and RO) independently reviewed all titles and abstracts identified (Figure 1) against the pre-specified eligibility criteria. Any disagreements were resolved by discussion with the other authors. All publications reporting a valid transfusion threshold RCT where inclusion criteria indicated that cardiovascular patients were included were considered. We contacted the authors of all eligible trials for which either cardiovascular subgroups or a high proportion of patients with cardiovascular disease were included. We requested data for the cardiovascular patients in these trials. For the trials that included patients both with and without cardiovascular disease, we looked at whether they stratified their randomisation by the presence or absence of cardiovascular disease.

We extracted data using a form piloted before the study. Two authors (AD and RO) independently extracted data on trial characteristics, primary and secondary outcomes, cardiac-specific morbidity and general morbidity. A third author (SS) checked for discrepancies between the independent data extraction, and disagreements were resolved by discussion between the three authors. Our primary outcome was mortality at 30 days. We also extracted mortality data at 60 days, intensive care and hospital mortality, and other mortality as defined by authors.

Data on cardiovascular events were categorised as Acute Coronary Syndrome (ACS), Acute Pulmonary Oedema (APO), peripheral ischaemia and thrombotic events wherever possible. The category of ACS included myocardial infarction (MI), acute coronary syndrome, and cardiac arrest.

Measures of general morbidity were use of packed RBCs, adverse transfusion reactions, incidence of in-hospital infections, measures of organ dysfunction, duration of ICU/hospital stay, invasive ventilation, haemodynamic support, and renal support.

Risk of bias assessment

We assessed the risk of bias using the method outlined in the Cochrane Collaboration Handbook for Systematic Reviews of Interventions(105). Risk of bias was assessed as high, low and unclear risk for each of: selection bias, performance bias, detection bias, attrition bias and reporting bias. We specifically assessed blinding for the outcomes of cardiovascular events.

Grading quality of evidence

We assessed the quality of evidence for mortality, acute coronary syndrome, and acute pulmonary oedema according to GRADE methodology for risk of bias, inconsistency, indirectness, imprecision, and publication bias; classified as very low, low, moderate, or high(106).

Data synthesis and analysis

All statistical analyses were performed using Review Manager 5 (RevMan)(107). Meta-analysis was undertaken where there were sufficient data. We used a random-effect model as we anticipated that there would be substantial clinical heterogeneity. We reported relative risk ratios (RR) for dichotomous outcomes, with 95% confidence intervals (CI). Where reported, we described non-parametric measures with median and interquartile ranges.

We included one cluster randomised trial(108). However, we had no information regarding which clusters the patients with cardiovascular disease were in, and the intraclass correlation coefficient (ICC) was 0.001 for mortality, suggesting that only 0.1% of the variance was due to the effect of the trial site, and 99.1% was due to differences between patients. We performed a sensitivity analysis without taking the clustering into account, and this made no difference to our results. We therefore included these data as unique patient data.

Assessment of heterogeneity

Assessment of clinical heterogeneity included consideration of participant characteristics (eg acute coronary syndrome vs chronic cardiovascular disease), and the clinical setting (critical care vs orthopaedics vs acute coronary syndrome). We undertook a subgroup analysis of patients with chronic cardiovascular disease, excluding

trials including patients with ongoing acute coronary syndromes. There was insufficient data to undertake the pre-planned subgroup analyses of critical care trials, or acute coronary syndromes.

We assessed statistical heterogeneity of treatment effects between trials using the Chi^2 test. We used the I^2 statistic to quantify the percentage of variability that was due to heterogeneity (we defined heterogeneity of >50% as moderate heterogeneity and >80% as substantial).

Patient Involvement

There was no direct patient involvement in this systematic review.

RESULTS

Search Results

The search retrieved 9,462 results (of which 283 were ongoing RCTs), which were reduced to 6,520 results once duplicates were removed. Previously screened references were removed and 3,955 titles and abstracts (3,832 completed trials and 123 ongoing RCTs), were screened for eligibility. Of these, 41 completed trials were eligible for full text screening (with five of the ongoing trials being potentially eligible for inclusion on their completion (109-113).

Thirty trials were ineligible for inclusion. Six trials were ineligible on the basis that they specifically excluded patients with signs or symptoms of heart disease, cardiac disease with New York Heart Association Class (NYHA) II or above, and American Society of Anaesthetists Class (ASA) II or worse (114-119). Two trials targeted pre-operative Haemoglobin S levels in sickle cell anaemia and were therefore not relevant (120, 121). Seven full text trials (122-128), and eight abstracts (109, 129-135), did not include cardiovascular disease as a baseline characteristic.

From the 41 potentially eligible trials, we were able to extract data on patients with cardiovascular disease from 7 trials (n=2,796) (108, 136-141). One of these trials published 30 day mortality data for patients with cardiovascular disease, and the authors responded to our request for further data, namely cardiovascular outcomes and general morbidity outcomes (n=32) (141). We were aware, from reported baseline demographic data, that patients with cardiovascular disease were included in eleven further trials (122, 142-151), but we were unable to extract any relevant data directly from the published text. Following contact with relevant authors, seeking any data they had on the patients with cardiovascular disease within their trials, we were able to include data from four trials into this review (122, 148-150). We did not contact the authors of the seven trials and eight abstracts which did not mention cardiovascular disease as a baseline characteristic in their trial (109, 123-135). Characteristics of all 29 eligible trials that are not included in this review can be found in the online data supplement (Table E1). In total, 11 transfusion threshold trials with patients with cardiovascular disease were included in this review (n=3,033).

Trial characteristics

The setting of the eleven included trials was varied: three orthopaedic trials (137, 148, 150), one trial with upper gastrointestinal bleeding (UGIB)(108), two trials with acute coronary syndrome/myocardial infarction (138, 139),

four trials in critical care (122, 140, 141, 149), and one trial in elective aortic and infra-inguinal revascularisation (136).

Definitions of cardiovascular disease differed between trials (Table E1). Other than one trial of elective aortic and infra-inguinal revascularisation (152), all trials included patients with the diagnosis of ischaemic heart disease, and all but the two acute MI trials (138, 139) included patients with congestive cardiac failure. Other trials also included risk factors for ischaemic heart disease including peripheral vascular disease (122, 136, 137, 140, 148, 150), cerebrovascular disease (122, 137, 148, 149), diabetes (148), and hypertension (148). Trials varied from all patients having cardiovascular disease (CVD) (136-139), to pre-defined CVD sub-groups (108, 140, 149), to high proportions of patients with CVD (122, 141, 148, 150). Included trials were both multicentre (n=7) (108, 137-141, 149) and single centre trials (n=4) (118, 122, 136, 150).

Red cell transfusion thresholds varied. The lowest restrictive threshold was 70g/l (122, 140, 141, 149) (n=274, from four trials), through 80g/l(108, 137, 138) (n=1,125, from three trials) and 90g/l(136) (n=50) to 97(150) (n=34). One trial transfused only with symptoms of anaemia (n=55)(148), and one used haematocrit concentration(139) (24%, n=24). Liberal thresholds also varied considerably: the most common thresholds were 90g/l(122, 141, 149, 153) (n=290, from four trials), and 100g/l (108, 136-138, 148) (n=1,221 from five trials). Other thresholds were Hb 113g/l (n=25) (150) and 30% haematocrit (n=21) (139). Leucocyte reduced RBCs were used in six out of the eleven trials (137-139, 141, 149, 150).

Through data extraction, we were able to identify unique data for patients with cardiovascular disease for 3,033 patients from eleven trials. The sample sizes of these trials varied from n=45 (139) to n=2015 (137). Of the 3,033 patients with cardiovascular disease, 1,514 were randomised to restrictive thresholds, and 1,519 to liberal thresholds. Six trials that included patients both with and without cardiovascular disease did not stratify their randomisation by the presence of absence of cardiovascular disease (108, 122, 140, 148-150). The characteristics of all included trials are described in full in Table 1.

Comparison of exposure to transfusion strategy

Duration of the intervention from randomisation: one trial maintained the haemoglobin threshold for one year post randomisation (148), one trial for 30 days (150), five trials until hospital discharge (108, 136-139), three trials until ICU discharge (122, 140, 149), and one trial for up to 14 days (141). The duration of exposure to the two strategies therefore varied considerably.

Exposure to allogenic blood: RBC transfusion requirements were extracted from six trials (136-139, 141, 148) (Table E3). For all six trials, patients in the restrictive arm were exposed to considerably less allogeneic blood than patients in the liberal arm. In the restrictive transfusion threshold arms between 20.4% (136) and 84.2% (148) of patients received no blood transfusions compared to a range of 0% (139, 148) to12% (136) for the liberal transfusion threshold arms. Among patients who did receive RBCs, the number of transfused units was lower in the restrictive transfusion threshold arms (range from median 0 (IQR 0,1) (137), to a mean of 1.6 units (SD 2.0) (139)) compared with the liberal transfusion threshold arms (range from a mean of 1.58 units (SD 1.13) (138) to a mean of 2.5 units (SD 1.3) (139)).

Effects on outcomes

Mortality

Data on mortality was available from all eleven trials. Thirty day mortality was given for all trials except one (108), who reported 28 day mortality. There were 144 deaths (9.5%) in the restrictive transfusion threshold arms, compared to 126 deaths (8%) in the liberal transfusion threshold arms (pooled effect estimate: RR 1.15, 95% CI 0.88 to 1.50, P=0.50, I^2=14%, 3033 patients, Figure 2). We performed a subgroup analysis, including only trials where the randomisation was stratified for cardiovascular disease (136-139, 141) and for this subgroup, the relative risk was 0.96 (95% CI 0.58 to 1.59, P=0.87, I^2=14%). The sensitivity analysis in which the two trials including patients with ACS were excluded (138, 139) supported the result of the primary analysis (RR 1.10, 95% CI 0.88 to 1.37). The GRADE quality of evidence was judged to be moderate (Table 2).

Two trials also presented mortality for all patients at 60 days (122, 137), and three at 90 days (148-150), however we were able to extract data on cardiovascular patients from only one trial (60 day mortality: Restrictive 66/1007 (6.6%) vs Liberal 76/998 (7.6%)) (137).

Adverse events: Cardiovascular

Nine trials presented data (2,609 patients) on new cardiovascular events (122, 136-141, 148, 149). The definition of MI varied between trials (Table E4). All trials except two (definition unclear) (140, 148) required ECG changes with a rise/fall of cardiac biomarkers using the Third Universal Definition of Myocardial Infarction (94). Five trials also required symptoms consistent with myocardial ischaemia (108, 122, 137, 139, 149). The diagnosis of MI was made by investigators in four trials (122, 137, 138, 149), clinicians in three trials (139, 141, 148), and was unclear in two trials (136, 140). The diagnosis was blinded in four trials (122, 137, 138, 149), unblinded in three

trials (139, 141, 148), and unclear in two trials (136, 140). The incidence of ACS (Figure 3) ranged from 0% (122, 148) to 20.4% (138) in the restrictive transfusion threshold arm and 0% (122, 139, 141, 148) to 11.1% (138) in the liberal transfusion threshold arm. There was evidence of an increased incidence of ACS in patients in the restrictive transfusion threshold arms compared with patients in the liberal transfusion threshold arms (RR 1.78, 95% CI 1.18 to 2.70, P=0.006, I^2= 0%, Figure 3A, Restrictive: 59 events/1319 patients vs Liberal: 32 events/1290 patients). This corresponds to 4.6 episodes of ACS per 100 patients when using restrictive strategies, and 2.7 per 100 patients when using liberal strategies. The number of patients that would need to be treated with a liberal transfusion strategy in order to prevent one episode of ACS is 52.

For the analysis of patients with APO, three trials had a higher incidence of acute pulmonary oedema in the liberal transfusion threshold arms (139, 140, 148), whereas one trial had a higher incidence in the restrictive transfusion threshold arm (138). There was no evidence of a different risk of APO in the restrictive transfusion threshold arms in comparison to the liberal transfusion threshold arms (RR 0.63, 95% CI 0.22 to 1.81, P=0.39, I^2=60%, Figure 3B. Restrictive: 24 events/309 patients vs Liberal: 47 events/340 patients)). Two trials reported no new episodes of APO (122, 148) and there was only one episode of APO in one trial (141). Cerebrovascular and thrombotic events were rare in both restrictive and liberal threshold groups and meta-analysis was not possible.

A sensitivity analysis excluding trials that did not stratify randomisation based on cardiovascular disease, found minimal impact on the estimates for the outcomes of ACS and acute pulmonary oedema. The GRADE quality of evidence was judged to be low for ACS mainly because of the serious risk of bias in outcome assessment (Table 2). Sensitivity analysis excluding the two ACS trials (138, 139) had minimal impact on the point estimates for this outcome of new ACS (RR 1.75, 1.10 to 2.80). The risk of ACS remained higher for the restrictive group on removal of the largest trial which had 2016 participants (137), RR 2.07 (1.02, 4.23).

Adverse events: General

Non-cardiovascular adverse events were reported across 8 trials (108, 136, 137, 139-141, 148, 150) (Table E5). These endpoints were described differently in each paper due to the different clinical settings and rationale of the trials.

Six trials reported hospital length of stay for patients with cardiovascular disease (136-141, 150). There was no significant difference between restrictive and liberal transfusion threshold arms (mean difference 1.24 days (95% CI -1.0 to 3.48, p=0.28, Figure E2)). Three trials reported in-hospital infection, but the number of events were small (138, 148, 150) (Table E2). One trial found no differences in organ support in a post hoc analysis of

cardiovascular patients (personal communication from author (149)), and there were no events classified as adverse transfusion reactions.

Risk of Bias

The risk of bias is summarised in Figures 2 and 3. The main category for high risk of bias was the lack of blinding of participants, clinical staff and research staff (identified in six trials (108, 137, 139-141, 149)). The diagnosis of cardiovascular events is difficult in many of the settings in which trials took place, such as during critical illness, increasing the risk of performance bias in conjunction with unblinded outcome assessors. Different definitions was another potential explanation for differing prevalence between trials (Table E4). Diagnosis of cardiovascular events was made by the investigators in five trials, and by unblinded clinicians in 3 trials (139, 141, 148). The criteria for myocardial infarction was clearly defined in seven trials (122, 136-139, 141, 149), and was unclear in two trials (140, 148). There was only one trial where both the definition and the outcome assessment were at high risk of bias (148), but no new cardiovascular events were diagnosed in this trial and its removal did not alter the analysis.

DISCUSSION

We identified data from eleven randomised trials that enrolled 3,033 patients with cardiovascular disease in whom mortality data were available at 30 days, and nine trials that enrolled 2,609 patients with cardiovascular disease in whom data regarding new cardiovascular events were available. A restrictive transfusion threshold was associated with an increased risk of ACS in patients with cardiovascular disease with low heterogeneity between trials (Moderate quality of evidence as assessed by GRADE). We found no evidence of a difference in 30 day mortality between restrictive and liberal transfusion threshold groups. There was no difference in the incidence of pulmonary oedema between the transfusion thresholds, but heterogeneity existed between trials and the GRADE quality of evidence was judged to be very low. There was no difference in hospital length of stay between restrictive and liberal transfusion strategies, and other outcomes were rare, with inadequate data for meta-analysis.

This is the first systematic review to specifically address clinical outcomes for patients with acute and chronic cardiovascular disease managed with restrictive or liberal transfusions not including patients undergoingcardiac surgery. Several well-conducted systematic reviews have been previously published, but these did not examine patient sub-groups with cardiovascular disease (71, 98, 99). The inclusion of heterogeneous populations in trials can mask potentially divergent effects in sub-populations (154), and effects may be amplified when trials are combined for meta-analyses. The 2012 Cochrane review recommended the use of a restrictive transfusion trigger,

but suggested caution in patients from high-risk groups such as acute coronary syndrome (99). Similar statements were made by both Holst (71) and Brunskill (98) in their systematic reviews of transfusion thresholds for sepsis and patients undergoing surgery for hip fracture respectively. Evidence is limited by the under-representation of patients with cardiovascular disease in many RCTs. For example, only 20% of patients enrolled in a large critical care trial had cardiovascular disease, compared with 29% of excluded patients (140). Similarly, only 14% of patients enrolled in a trial in septic shock had cardiovascular disease (149), whereas observational trials suggest around 25-30% of critical care populations may have co-existing cardiac disease (95, 155).

The previous reviews in heterogeneous populations suggest overall trends toward lower 30 day mortality with restrictive practices (range of RR 0.85, 95% CI 0.70-1.03 (99) to 0.92, 95% CI 0.67-1.26 (98)), whereas the effect we observed in patients with cardiovascular disease was in the direction favouring liberal transfusion, but without statistical significance (RR 1.10, 95% CI 0.84 to 1.44). We specifically excluded trials in cardiac surgery from our review, because this is a distinct group of patients whose cardiovascular risk has been significantly altered by their procedure. A recent large multicentre RCT in cardiac surgery (156) found no difference in a composite morbidity outcome, but 90-day mortality rate was significantly higher in the restrictive transfusion threshold group compared with the liberal group. A recent systematic review and meta-analysis restricted to cardiac surgery trials also found increased mortality with restrictive transfusion thresholds (101). Another systematic review (157) of perioperative RCTs of transfusion practice (including cardiac surgery) also demonstrated higher mortality with a restrictive transfusion threshold trigger, although the prevalence of cardiovascular disease in these trials was uncertain. These data suggest that the presence of cardiovascular disease may significantly modify the effect of transfusion practice on clinical outcomes, and highlight the need for better evidence for this prevalent patient group.

We found that new onset ACS occurred more frequently with restrictive transfusion threshold practices. The pooled estimates were 2.7% for liberal versus 4.6% for restrictive practices (number needed to treat approximately 52 to prevent an ACS with more liberal transfusion). The variation in patient populations, transfusion strategies compared, and method of ascertaining ACS create substantial uncertainty in these estimates, but the heterogeneity between trials was low. The estimate of effect was the same when the largest trial was removed. Importantly, for the majority of included cases the restrictive transfusion threshold was 80g/l versus a liberal threshold of 100g/l. These findings suggest that a transfusion threshold of 70g/l, which is widely recommended as the "default" threshold, may not be as safe as higher thresholds for preventing ACS in patients with cardiovascular disease. The safest haemoglobin threshold is uncertain and may be patient specific, but we have shown potential for harm with

restrictive triggers below 80g/l. Further trials are needed to inform us on the optimal transfusion strategy in patients with cardiovascular disease. Myocardial injury could impact on other important clinical outcomes such as length of hospital stay, quality of life, longer term mortality, and healthcare costs but few trials measured these outcomes. These outcomes, together with cost effectiveness, should be included in future research particularly as the cost of blood transfusions is relatively low and even in the liberal transfusion threshold arm in this review patients typically received only two to three units. Our review highlights the variability in diagnostic definitions of ACS and the potential for ascertainment bias in clinical trials where blinding of intervention groups is difficult. This resulted in the GRADE assessment as low for the evidence quality, and highlight the need for further high quality research.

We found no effect on APO, but the numbers of trials and patients in whom this outcome was reported was small and there was heterogeneity in the findings. APO can result from multiple causes, including Transfusion Associated Circulatory Overload (TACO), and the potential for an effect in a different direction from ACS made it important to consider these outcomes separately rather than include them as a composite. Future trials should standardise diagnostic methods for both ACS and APO and attempt to blind outcome assessors to group allocation.

Our review has a number of limitations. There was clinical diversity between trial populations, for example between orthopaedic surgery and critical care. The risk-benefit balance may vary between clinical situations, for example as a result of the degree and duration of physiological stress. The restrictive and liberal transfusion thresholds varied between trials, and the cut-off values actually overlapped (restrictive threshold 70-97g/l; liberal threshold 90-113g/l), which reduces the validity of pooling data across all trials. Exposure to anaemia would have been considerably longer in the four ICU trials than in the four surgical trials, in which exposure to anaemia would have been relatively short. Definitions of cardiovascular disease varied, and inclusion criteria for some trials were restricted to ischaemic heart disease or acute coronary syndrome. However, the direction of effect was consistently in favour of a liberal transfusion threshold for reducing new ACS events across the trials. Finally, not all authors responded to our request for data for cardiovascular patients in their trials, which reduced the precision of our point estimates.

In conclusion, this review of available evidence suggests that for anaemic patients with cardiovascular disease the use of restrictive haemoglobin thresholds for blood transfusion (typically 70-80g/l) is associated with higher rates of ACS than more liberal thresholds (typically 90-100g/l). No effects on mortality or other important outcomes were demonstrated. The currently available quality of evidence for all outcomes is low. These data support the

use of a more liberal transfusion threshold (greater than 80g/l) for patients with both acute and chronic cardiovascular disease, until adequately powered high quality randomised trials have been undertaken in this patient population.

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Author Contributions: Dr Docherty had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Docherty, O'Donnell, Brunskill, Trivella, Walsh, Stanworth

Acquisition, analysis, or interpretation of the data: Docherty, O'Donnell, Brunskill, Doree, Walsh, Stanworth, Holst, Parker, Gregersen, Almeida

Drafting of the manuscript: Docherty, O'Donnell, Brunskill, Walsh, Stanworth

Statistical Analysis: Docherty, Trivella

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Study supervision: Walsh, Stanworth

Dr Annemarie Docherty affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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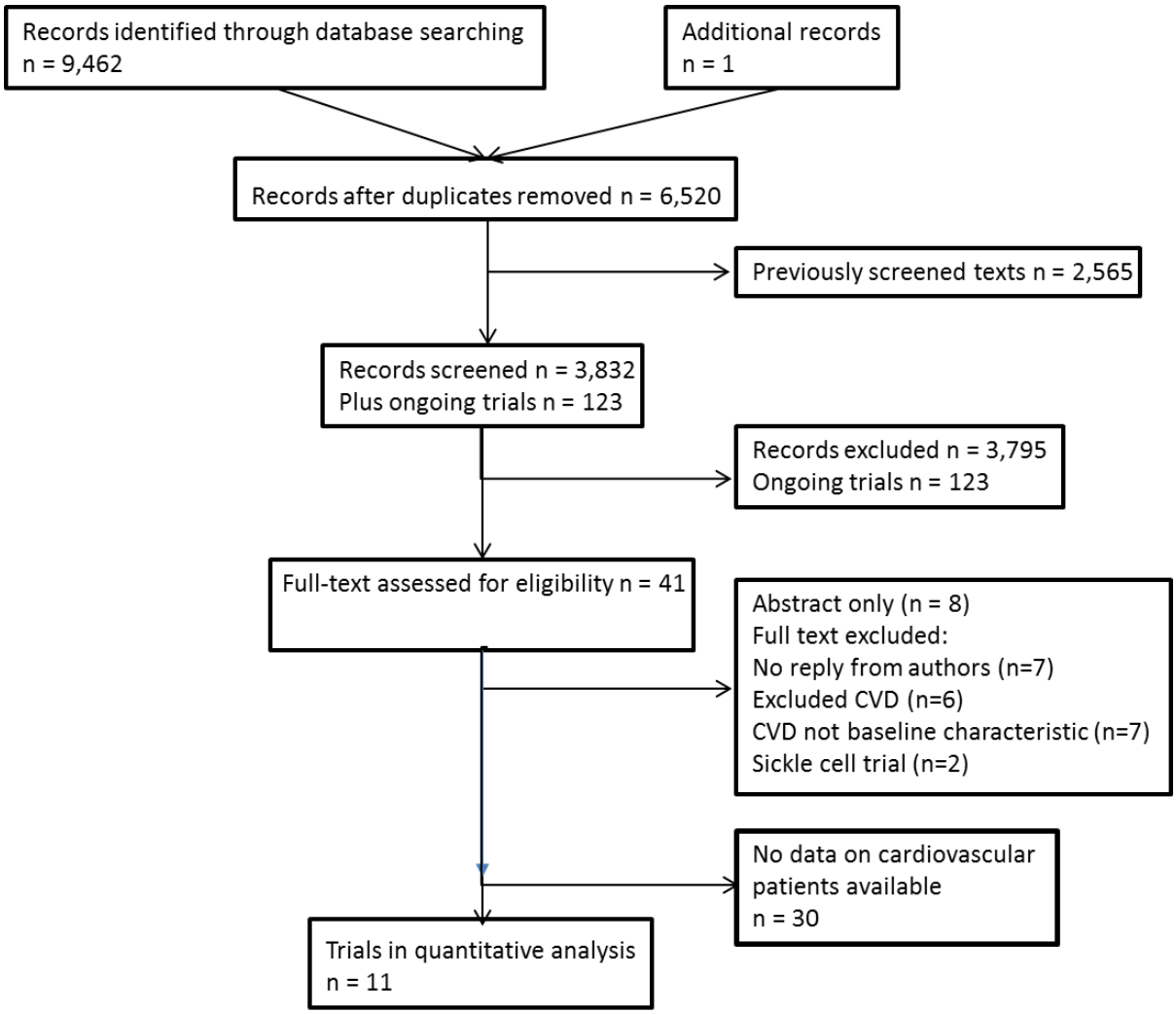

Figure 1: ***PRISMA Flow Diagram***

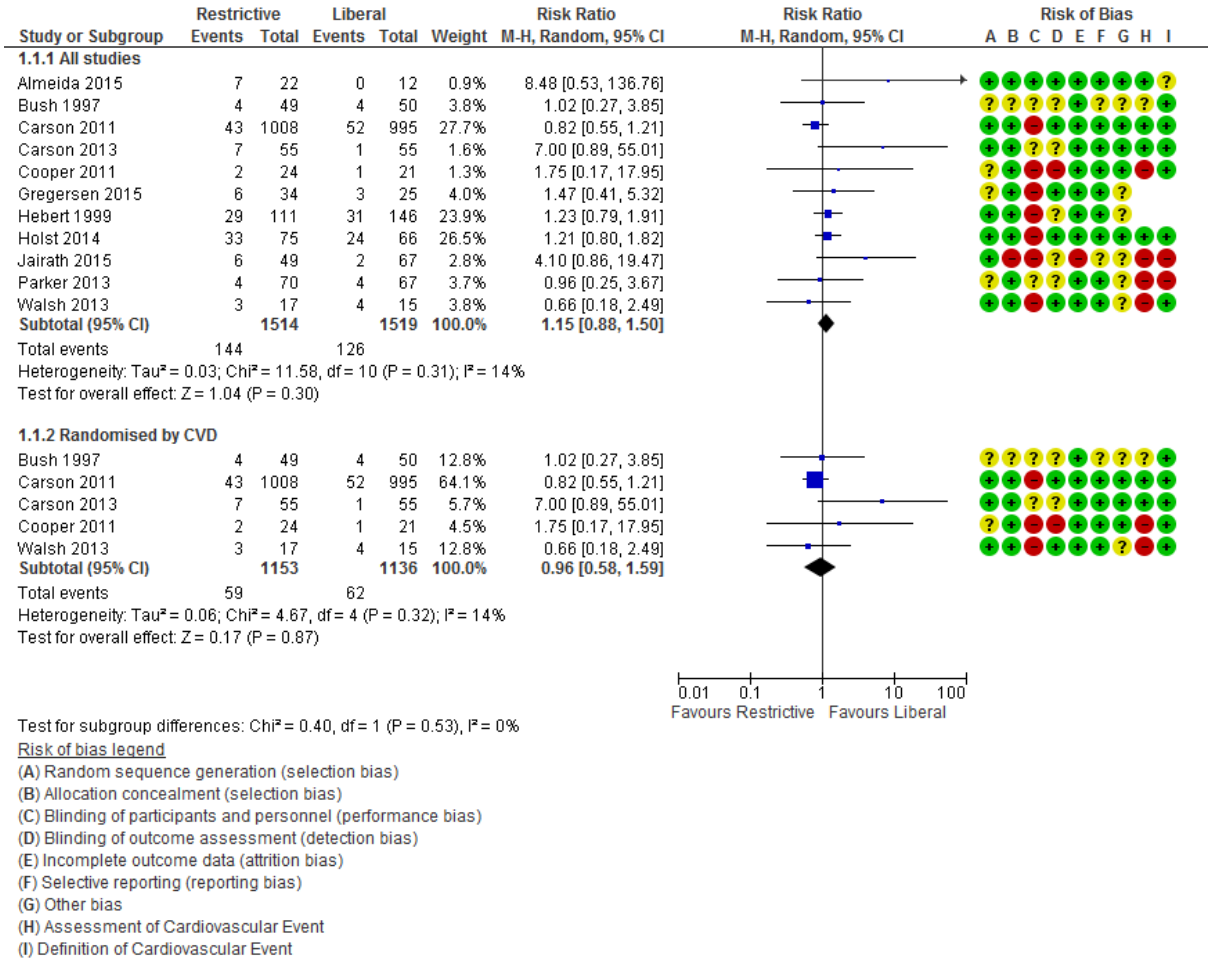

Figure 2: ***Forest plot of Risk Ratios for 30 day mortality with risk of bias assessment for each study. 1.1.1: all studies; 1.1.2: studies that randomised by cardiovascular disease. *Jairath: Additional risk of bias assessed as to completeness of patients recruited into clusters. This was graded as low risk.***

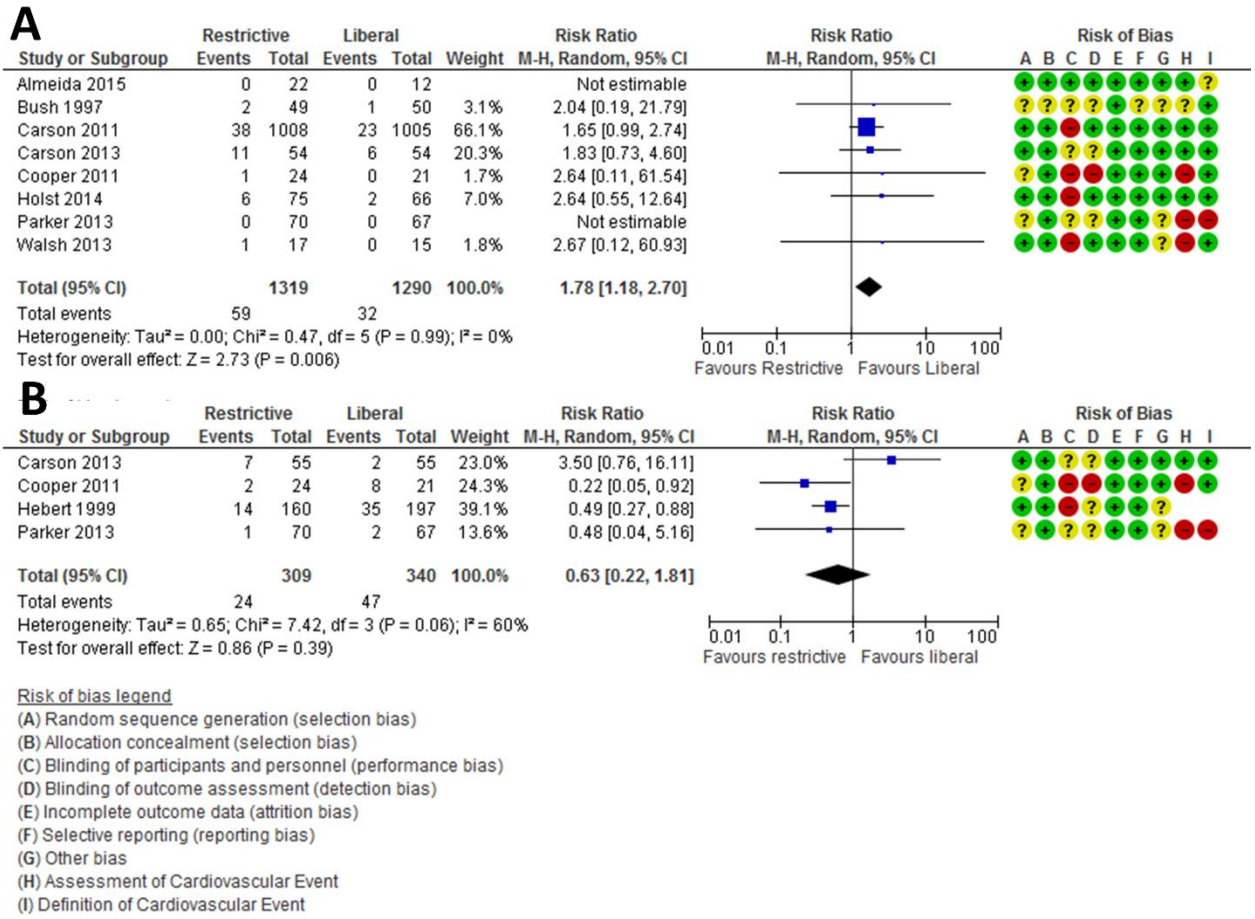

Figure 3 Forest plot with Risk Ratios for Adverse Cardiovascular Events:

A: Myocardial infarction, Acute Coronary Syndrome, Cardiac Arrest. Risk of bias assessment included for each study.

B: Acute Pulmonary Oedema

Table 4: Characteristics of included trials contributing to data-analysis. S: Single-centre, M: Multi-centre trial. CV: Cardiovascular; MI Myocardial Infarction; GI: GastroIntestinal

Author	**Journal**	**Clinical Setting** (*leucodepleted)	**Trigger**	**Total n**	**CV n (%)**	**Primary endpoint**
Almeida 2013 Brazil (S)	Critical Care	Oncology	Restrictive (R) 70g/l	101	22 (21.8)	Composite death or severe complications
			Liberal (L) 90g/l	97	12 (12.4)	
Bush 1997 USA (S)	American Journal of Surgery	Elective vascular surgery	R: 90g/l	50	50 (100)	Myocardial ischaemia, myocardial infarction, death
			L: 100g/l	49	49 (100)	
Carson 2011 USA/Canada (M)	NEJM	Hip fracture patients with Cardiovascular disease (CVD) or risk factors for CVD*	R: 80g/l or symptoms of anaemia	1009	1009 (100)	60 day mortality Walk unaided
			L: 10g/dl	1007	1007 (100)	
Carson 2013 USA (M)	American Heart Journal	Symptomatic Coronary Artery Disease*	R: 8g/dl or symptoms of anaemia	55	55 (100)	Composite: all cause mortality, myocardial infarction or unscheduled coronary revascularization
			L: 10g/dl	55	55 (100)	
Cooper 2011 USA (M)	American Journal of Cardiology	Acute MI*	R: hct <24%,	24	24 (100)	Composite: In-hospital death, recurrent MI, new or worsening congestive heart failure
			L: hct <30%	21	21 (100)	
Gregersen 2015 Denmark (S)	Acta Orthop.	Frail elderly hip fracture patients*	R: 97g/l	116	34 (29.3)	Recovery from physical disabilities
			L: 113g/l	111	25 (22.5)	
Hebert 1998 Canada (M)	NEJM	Critical care	R: 70g/l	418	160 (38.2)	30 day mortality
			L: 90g/l	420	197 (46.9)	
Holst 2014 Scandinavia (M)	NEJM	Critical care*	R: 70g/l	502	75 (14.9)	90 day mortality
			L: 90g/l	496	66 (13.3)	
Jairath 2015 UK (M)	Lancet	Upper GI haemorrhage	R: 80g/l	403	61 (15%)	Feasibility
			L: 100g/l	533	76 (14%)	
Parker 2013 UK (S)	Injury	Hip fracture patients	R: definite symptoms of anaemia	100	50 (50.0)	30d mortality
			L: raise Hb to at least 10.0g/dl	100	37 (37.0)	
Walsh 2013 UK (M)	Critical Care Medicine	Critical care*	R:70g/l	51	17 (33.3)	Feasibility: Difference in mean Hb during intervention period.
			L: 90g/l,	49	15 (30.6)	

Table 5: Summary of findings for all trials including GRADE quality of evidence assessment.

RR – relative risk. 1. Not all participants/clinicians blinded; 2. definition varied between studies; 3. Not all investigators blinded; 4. substantial heterogeneity; 5. low numbers

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Restrictive**	**Liberal Transfusion Threshold**	**Relative (95% CI)**	**Absolute (95% CI)**		
30 Day Mortality												
11	randomised trials	serious [1]	not serious	not serious	not serious	none	144/1514 (9.5%)	126/1519 (8.3%)	**RR 1.15** (0.93 to 1.43)	12 more per 1000 (from 6 fewer to 36 more)	⊕⊕⊕◯ MODERATE [1]	
								6.0%		9 more per 1000 (from 4 fewer to 26 more)		
Cardiovascular events												
8	randomised trials	very serious [1 2 3]	not serious	not serious	not serious	none	59/1319 (4.5%)	32/1290 (2.5%)	**RR 1.78** (1.18 to 2.70)	19 more per 1000 (from 4 more to 42 more)	⊕⊕◯◯ LOW [1 2 3]	
								1.0%		8 more per 1000 (from 2 more to 17 more)		
Acute Pulmonary Oedema												
4	randomised trials	very serious [1 2 3]	serious [4]	not serious	serious [5]	none	24/309 (7.8%)	47/340 (13.8%)	**RR 0.58** (0.36 to 0.92)	58 fewer per 1000 (from 11 fewer to 88 fewer)	⊕◯◯◯ VERY LOW [1 2 3 4 5]	
								10.7%		45 fewer per 1000 (from 9 fewer to 68 fewer)		

Online Data Supplement

Table E1: Authors' definition of Cardiovascular Disease

Author	Definition of Cardiovascular disease
Almeida 2013	Arterial coronary artery disease, congestive heart failure, cerebrovascular disease, peripheral arterial disease
Blair 1986	Not discussed
Bush 1997	Patients undergoing elective aortic or infrainguinal arterial reconstruction
Carson 2011	history of ischaemic heart disease, electrocardiographic evidence of previous myocardial infarction, a history or presence of congestive heart failure or peripheral vascular disease, or a history of stroke or transient ischaemic attack
Carson 2013	ST segment elevation myocardial infarction, Non ST segment elevation myocardial infarction, Unstable angina (symptoms of cardiac ischemia at rest with at least one episode lasting 10 minutes AND ST-segment depression of 0.01 mV or more or transient [b30-minute] ST-segment elevation of 0.1 mV or more in two or more contiguous leads), OR prior documented coronary artery disease (myocardial infarction, percutaneous cardiac intervention, coronary artery bypass graft surgery), or age N55 with diabetes mellitus or peripheral arterial disease and no biomarker elevation Stable coronary artery disease undergoing a cardiac catheterization (presence of coronary artery disease (one cardiac artery with at least 70% obstruction by visual inspection based on cardiac catheterization or undergoing a percutaneous cardiac intervention,)during index admission
Cooper 2011	AMI was defined as ischaemic-type chest discomfort lasting 30 minutes and associated with a creatine kinase-MB (CK-MB) or cardiac troponin level above the upper limit of normal (determined locally).
Gregersen 2015	Charlson Comorbidity Index on ICD-10. If a patient was diagnosed with one of the first two boxes (myocardial infarct or congestive cardiac insuffiency/ I21; I22; I23; I50; I11.0; I13.0; I13.2)
Jairath 2015	History of ischaemic heart disease
Hebert 1999	primary or secondary ICU admission diagnosis of a cardiovascular disease, as well as those patients with cardiac disease as an important comorbid illness defined as New York Heart Association class III or IV. As a second step, we examined all patients who were known to have ischemic heart disease. The diagnosis most responsible for the patient's ICU admission was recorded. As many as three secondary diagnoses and up to eight separate comorbid conditions were identified. The cardiovascular disease category included all diagnoses related to ischemic heart disease (myocardial infarct, angina, congestive heart failure, and cardiogenic shock), rhythm disturbances, cardiac arrest, other forms of shock, uncontrolled hypertension, and cardiac and vascular surgical procedures such as abdominal aortic aneurysm repair and peripheral vascular surgical procedures.
Holst 2014	history of myocardial infarction, any history of stable or unstable angina pectoris, previous treatment with nitrates, percutaneous coronary intervention, coronary-artery bypass grafting or noncoronary vascular interventions, any history of chronic heart failure [defined as New York Heart Association class III or IV], or any history of cerebral infarction or transitory cerebral ischemia
Parker 2013	Hypertension Angina Previous myocardial infarction Previous congestive cardiac failure Other cardiac disease
Walsh 2013	Ischaemic heart disease was defined as evidence from the patient's records of a previous history of angina, previous myocardial infarction, or chronic cardiac failure at the time of randomization.

Table E2: Characteristics of all eligible blood transfusion trials not included in quantitative analysis

Author	Journal	Clinical Setting	Trigger	Total n	CV n (%)	Primary endpoint	Reason for exclusion
Bergamin 2014 Brazil (S)	Critical Care	Critical Care	R: 70g/l	73		28 day mortality	CVD not baseline characteristic
			L: 90g/l	63			
Blair 1986 UK (S)	British Journal of Surgery	GI haemorrhage	R: 80g/l or shock	26		Clotting, death	CVD not baseline characteristic
			L: 2 units	24			
Carson 1998 USA/Scotland (M)	Transfusion	Hip fracture patients	R: 80g/l or symptoms of anaemia	42	19 (45.2)	60 day mortality	no reply from authors
			L: 100g/l	42	19 (45.2)		
Colomo 2008 Spain (S)	Hepatology (abstract)	GI haemorrhage in cirrhosis	R: 70g/l	109		mortality	no further information available
			L: 90g/l	105			
Fan 2014 China (S)	Archives of Gerontology and Geriatrics	elective Total hip replacement	R: 80g/l or symptoms of anaemia	41	IHD: 9, BP: 52, CVA:4, CCF: 2	Postoperative Delirium (POD)	No reply from authors
			L: 100g/l	42	IHD: 10, BP: 57, CVA: 3, CCF: 3		
Fortune 1987 USA (S)	Journal of Trauma	Traumatic patients with class 3-4 haemorrhagic shock	R: hct near 30%	12		Metabolic stability	CVD not baseline characteristic
			L: hct near 40%	13			
Foss 2009 Denmark (S)	Transfusion	Hip fracture patients	R: 80g/l	60	28 (46.7)	Postoperative functional mobility	No reply from authors
			L: 100g/l	60	21 (35.0)		
Grover 2006 UK (S)	Vox Sanguinis	orthopaedic surgery	R: 80g/l	109	angina: 6 (5.5) MI: 6 (5.5)	Silent myocardial ischaemia	ECG abnormalities excluded
			L: 100g/l	109	angina: 8 (7.3) MI: 6 (5.5)		
Haberkern 1997 USA (M)	Blood	sickle cell surgery	R: conservative	110		Sickle cell events	pre-operative HbSS transfusion target
			L: aggressive	120			
			Not randomised	134			
Hebert 1995 Canada (M)	JAMA	Critical care	R: 70g/l	33	5 (15.2)	Feasibility Mortality	No reply from authors
			L: 90g/l	36	4 (11.1)		
Hochain 1996	Gut (abstract)	variceal bleeding	R: PCV 25	43		Rebleeding	CVD not mentioned
			L: PCV 32	47			
Park H 2008 South Korea (S)	Cancer Chemo and Pharm	Gastric oncology	R: 100g/l			Response Quality of Life	CVD not mentioned
			L: 120g/l				
Koshy 1988 USA (S)	NEJM	sickle cell pregnancy	R:as required	36		Perinatal mortality	HbSS transfusion target
			Prophylactic transfusion	36			
Liu 2015 China (S)	Chinese Medical Journal	Emergency surgery	R: illness severity score	33		Feasibility	Exc: patients with coronary heart disease
			L: standard care	32			
Lotke 1999 USA (S)	Journal of Arthroplasty	Total knee arthroplasty	R: 90g/l	62		Not explicit	CVD not baseline characteristic

Author	Journal	Clinical Setting	Trigger	Total n	CV n (%)	Primary endpoint	Reason for exclusion
			L: 2 units starting in recovery room	65		Hb, LOS, wellbeing	
Mazza 2015 Brazil (M)		Critical Care	R: 70g/l	22	7 (31.8)	Effect of transfusion on lactate and SvO_2	No reply from authors
			L: 90g/l	24	10 (41.7)		
Nielsen 2012	Transfusion medicine	Spinal surgery	R: 73g/l	25		subcutaneous oxygen tension	Exc: cardiac disease NYHA II or above
			L: 89g/l	23			
Nielsen 2014 Denmark (S)	BMC Anesthesiology	Elective hip revision	R: 73g/l	33	5 (15.1)	Timed Up and Go-test	No reply from authors
			L: 89g/l	33	7 (21.2)		
Palmer 1998 Scotland (S)	Transfusion Medicine (abstract)	hip fracture					abstract unavailable
Prick 2013 Netherlands (M)	BJOG	postpartum haemorrhage Hb 48-79g/l	R: no transfusion	262		Physical fatigue	Excluded ASA 2-4 patients
			L: transfusion	259			
Robertson 2014 USA (M)	JAMA	Traumatic brain injury	R: 70g/l	99		Glasgow Outcome Scale	CVD not baseline characteristic
			L: 100g/l	101			
So-Osman 2004 Netherlands (S)	Vox Sanguinis (abstract)	hip/knee surgery	unclear			Blood use	CVD not mentioned
So-Osman 2010 Netherlands (M)	Vox Sanguinis	Elective Total knee or hip replacement	R: stratified by risk	299	205 (68.6)	Hospital LOS	30 day mortality not measured
			L: standard care	304	211 (69.4)		
Villanueva 2013 Spain (S)	NEJM	Upper GI bleeding	R:70g/l	444		Mortality at 45days	CVD not baseline characteristic, many CV patients excluded
			L: 90g/l	445			
Villarejo 1999 Spain (S)	Acta Gastroenterol Latinoam.	GI haemorrhage	R: hct 21%				CVD not mentioned
			L: hct 28%				
Webert 2008 Canada (M)	Transfusion	Leukaemia	R: 80g/l	29		Bleeding	CVD patients excluded
			L: 120g/l	31			
Weiss 1982 USA (S)	Lancet (letter)	Acute leukaemia	R: as needed	12		Marrow recovery	CVD not baseline characteristic
			L: 120g/l	12			
Wu 2011 China (S)	Intensive Care Medicine (abstract only)	liver transplant	R: 70g/l	112		30 day mortality	CVD not mentioned
			L: 100g/l	114			
Zheng 2013 China (S)	Experimental and Therapeutic Medicine	Orthopaedic surgery elderly patients	Standard	52		blood transfusion effectiveness	CVD patients excluded
			Goal directed	54			
Zygun 2009 UK (S)	Critical Care Medicine	traumatic brain injury	R: 8g/dl			Change in brain tissue oxygenation	CVD not baseline characteristic
			Mid: 9g/dl				
			L: 10g/dl				

Table E3: Patients receiving allogenic blood. Mean (SD) or Median (IQR) as quoted in orginal paper

	Restrictive trigger	Pts receiving blood	%	No. units transfused	Liberal trigger	Pts receiving blood	%	No. units transfused
Bush 1997	90g/l n=49	39	79.6	1.5 (1.7)	100g/l n=50	44	88.0	2.4 (2.5)
Carson 2011	80g/l,Sx anaemia n=1008	413	41.0	0 (0,1)	100g/l n=1007	973	96.6	2(1,2)
Carson 2013 Acute MI	80g/l n=55	15	27.3	0.49 (1.03)	100g/l n=55	52	94.5	1.58(1.13)
Cooper 2011	Hct <24% n=24	13	54.2	1.6 (2.0)	Hct <30% n=21	21	100.0	2.5 (1.3)
Parker	Sx anaemia n=70	11	15.7	0	100g/l n=67	67	100.0	2 (2,2)
Walsh	70g/l n=17	12	70.6	1.5 (0.25,3.5)	90g/l n=15	15	100.0	2 (1,8)

Table E4: Diagnosis of Cardiovascular events:

I: diagnosed by Investigator, C: diagnosed by Clinical team, U: unclear

	Blinded Y/N	Diagnosis
Almeida 2015 (I)	Y	Clinical symptoms suggestive of myocardial ischaemia with >=1: increase or decrease in cardiac troponin I (>=1 value >99th centile URL) ECG changes: new Q waves, ST-elevation, new LBBB image based evidence of new loss of viable myocardium
Bush 1997 (U)	?	New Q waves and/or CPK elevation (MB fraction >5ng/ml and relative index >2.0)
Carson 2011 (I)	Y	Detection of rise or fall of cardiac troponin I with at least one value above the 99th percentile of the upper reference limit in the context of myocardial ischemia and at least one of the following: Symptoms of myocardial ischemia; New ECG changes indicative of ischemia (eg ST-T changes or new left bundle branch block [LBBB]) or development of pathological Q waves Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality Autopsy evidence of recent myocardial necrosis
Carson 2013 (I)	Y	Rise and/or fall of cardiac biomarkers (preferably troponin) together with evidence of myocardial ischemia and either symptoms of ischemia or electrocardiogram changes indicative of new ischemia. Unstable angina was defined as: (1) the absence of elevated cardiac biomarkers and (2) presence of ischemic symptoms or electrocardiogram changes indicative of ischemia or (3) chest pain or angina equivalent leading to a coronary artery intervention (e.g., coronary angioplasty) and (4) hospitalization. Congestive heart failure required at least one of the following symptoms or signs, new or worsening including: dyspnea at rest, orthopnea, or paroxysmal nocturnal dyspnea, AND radiological evidence of heart failure or worsening heart failure AND additional/increased therapy.
Cooper 2011 (C)	N	Recurrent ischemic chest discomfort, new ischemic electrocardiographic changes, and CK-MB increase above the upper limit of normal and increased by 50% over the previous value. For patients with percutaneous coronary intervention 24 hours previously, CK-MB >3 times the upper limit of normal and increased by >50% over the previous value was required. For patients with coronary artery bypass grafting surgery 24 hours previously, CK-MB >5 times the upper limit of normal and increased by >50% over the previous value was required. New or worsening HF was defined as 1 of the following occurring >6 hours after randomization: cardiogenic shock or a physician's decision to treat HF with an intravenous diuretic or intravenous vasoactive drug and evidence of pulmonary vascular congestion.
Hebert 1999 (I)	?	Unclear
Holst 2014 (I)	Y	Symptoms, electrocardiographic signs, or elevated biomarker levels resulting in an intervention
Parker 2013 (C)	N	Unclear
Walsh 2013 (C)	N	Troponin rise, new ECG change

Table E5: Adverse events – General. Mean (SD)

	Restrictive trigger	**Change in MODS**	**Length of ICU stay (d) LOS Hospital (d)**	**In-hospital infections**	**Liberal trigger**	**MODS**	**Length of ICU stay (d) LOS Hospital (d)**	**In-hospital infections**
Bush 1997	90g/l		4 (8) days 11 (9) days		100g/l		4 (4) days (p>0.6) 10 (6) days (p>0.6)	
Carson 2011 USA (n=1220) Canada (n=791)	80g/l, Sx anaemia		USA 4.0 (3.9) Canada 12.7 (9.5)				USA 3.7 (3.4) Canada 12.0 (9.3)	
Carson 2013 Acute MI	80g/l			2	100g/l			2
Cooper 2011	Hct <24%		4.3 (3.3) (CCU) 10.4 (7.2)		Hct <30%		3.4 (2.3) (CCU) 4.3 (3.3)	
Gregersen	97g/l		Hosp 7.8 (5.0)	13	113g/l		Hosp 8.4 (8.0)	24
Hebert 1998	70g/l	0.23 +/- 4.2	9.3 (9.7) 28.76 (19.5)		100g/l	1.3 +/- 4.4	10.4 (10.3) 30.6 (18.8)	
Parker	Sx anaemia			3	100g/l			5
Walsh	70g/l		36.5 (26.7) 53.3 (40.1)		90g/l		25.6 (18.1) 36.3 (28.3)	

Figure Legends:

Figure E1: Forest plot with mean difference for hospital length of stay.

SEARCH STRATEGIES

CENTRAL

#1 MeSH descriptor: [Erythrocyte Transfusion] explode all trees
#2 MeSH descriptor: [Blood Transfusion] this term only
#3 ((blood or erythrocyte* or "red cell*" or "red blood cell*" or RBC*) near/5 (transfus* or unit* or infus* or therap*))
#4 ((red cell* or RBC* or erythrocyte* or red blood cell* or whole blood or transfus*) near/5 (use* or usage* or utiliz* or utilis* or requir* or need* or administ* or replac* or support* or strateg* or management or practic* or indicat* or criteri* or standard* or program*)):ab
#5 ((red cell* or RBC* or erythrocyte* or blood or transfus*) and (use* or usage* or utiliz* or utilis* or requir* or need* or administ* or replac* or support* or strateg* or management or practic* or indicat* or criteri* or standard* or program*)):ti
#6 (leukodeplet* or leukoreduc* or leucodeplet* or leucoreduc* or leukofiltrat* or leucofiltrat*):ti
#7 ("allogeneic blood" or (unit* near/2 blood) or "allogenic blood" or (blood near/2 exposure) or "donor blood" or "blood product*" or "blood component*" or "blood support")
#8 (hemotransfus* or haemotransfus* or hypertransfus* or hemotherap* or haemotherap*)
#9 (red cell* or erythrocyte* or blood or RBC*) and transfus*:ti
#10 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9
#11 MeSH descriptor: [Blood Component Transfusion] this term only
#12 MeSH descriptor: [Exchange Transfusion, Whole Blood] explode all trees
#13 MeSH descriptor: [Plasma Exchange] explode all trees
#14 MeSH descriptor: [Platelet Transfusion] explode all trees
#15 MeSH descriptor: [Leukocyte Transfusion] explode all trees
#16 #12 or #13 or #14 or #15
#17 #11 not #16
#18 ("red cell*" or "red blood cell*" or erythrocyte* or RBC*)
#19 MeSH descriptor: [Erythrocytes] this term only
#20 #18 or #19
#21 #17 and #20
#22 #10 or #21
#23 MeSH descriptor: [Hematocrit] this term only
#24 ((h?emoglobin or h?emocrit* or HB or HCT) near/5 (level* or concentration* or target* or maintain* or rais* or higher or lower or greater or above or below or equal or transfus*))
#25 #23 or #24
#26 #22 and #25
#27 ((transfus* or "red cell*" or "red blood cell*" or RBC*) near/10 (trigger* or thresh?old* or target* or restrict* or liberal* or aggressive* or conservative* or prophylactic* or limit* or protocol* or policy or policies or practic* or standard*))
#28 ((transfus* or "red cell*" or "red blood cell*" or RBC* or h?ematocrit*) and (level* or critical* or intensive* or h?emorrhag* or bleed*) or hypertransfus*):ti
#29 #26 or #27 or #28 Publication Year from 2008 to 2014

MEDLINE (OVID)

1. randomized controlled trial.pt.
2. controlled clinical trial.pt.
3. randomi*.tw.
4. placebo.ab.
5. clinical trials as topic.sh.
6. randomly.ab.
7. groups.ab.
8. trial.ti.
9. or/1-8
10. exp animals/ not humans/
11. 9 not 10
12. BLOOD TRANSFUSION/
13. ERYTHROCYTE TRANSFUSION/

14. ((blood or erythrocyte* or red cell* or red blood cell* or RBC*) adj5 (transfus* or infus* or unit* or therap*)).ti,ab.
15. ((red cell* or RBC* or erythrocyte* or red blood cell* or whole blood or transfus*) adj5 (use* or usage* or utiliz* or utilis* or requir* or need* or administ* or replac* or support* or strateg* or management or practic* or indicat* or criteri* or standard* or program*)).ab.
16. ((red cell* or RBC* or erythrocyte* or blood or transfus*) and (use* or usage* or utiliz* or utilis* or requir* or need* or administ* or replac* or support* or strateg* or management or practic* or indicat* or criteri* or standard* or program*)).ti.
17. (allogeneic blood or (unit* adj2 blood) or allogenic blood or (blood adj2 exposure) or donor blood or blood product* or blood component* or blood support).ti,ab.
18. (hemotransfus* or haemotransfus* or hypertransfus* or hemotherap* or haemotherap*).tw.
19. (red cell* or erythrocyte* or blood or RBC*).tw. and transfus*.ti.
20. or/12-19
21. BLOOD COMPONENT TRANSFUSION/
22. EXCHANGE TRANSFUSION, WHOLE BLOOD/ or PLASMA EXCHANGE/ or PLATELET TRANSFUSION/ or exp LEUKOCYTE TRANSFUSION/
23. 21 not 22
24. ERYTHROCYTES/
25. (red cell* or red blood cell* or erythrocyte* or RBC*).tw.
26. 24 or 25
27. 23 and 26
28. 20 or 27
29. *HEMATOCRIT/
30. ((h?emoglobin or h?ematocrit* or HB or HCT) adj5 (level* or concentration* or target* or maintain* or rais* or higher or lower or greater or above or below or equal or transfus*)).tw.
31. 29 or 30
32. 28 and 31
33. ((transfus* or red cell* or red blood cell* or RBC*) adj10 (trigger* or thresh?old* or target* or restrict* or liberal* or aggressive* or conservative* or prophylactic* or limit* or protocol* or policy or policies or practice* or standard*)).tw.
34. (((transfus* or red cell* or red blood cell* or RBC* or h?ematocrit*) and (level* or critical* or intensive* or h?emorrhag* or bleed*)) or hypertransfus*).ti.
35. 32 or 33 or 34
36. 11 and 35

EMBASE (OVID)
1. Randomized Controlled Trial/
2. Randomization/
3. Single Blind Procedure/
4. Double Blind Procedure/
5. Crossover Procedure/
6. Placebo/
7. exp Clinical Trial/
8. Prospective Study/
9. (randomi* or double-blind* or single-blind* or RCT*).tw.
10. (random* adj2 (allocat* or assign* or divid* or receiv*)).tw.
11. (crossover* or cross over* or cross-over* or placebo*).tw.
12. ((treble or triple) adj blind*).tw.
13. or/1-12
14. Case Study/
15. case report*.tw.

16. (note or editorial).pt.
17. or/14-16
18. 13 not 17
19. (animal* or cat or cats or dog or dogs or pig or pigs or sheep or rabbit* or mouse or mice or rat or rats or feline or canine or porcine or ovine or murine).ti.
20. 18 not 19
21. limit 20 to embase
22. BLOOD TRANSFUSION/
23. ERYTHROCYTE TRANSFUSION/
24. ((blood or erythrocyte* or red cell* or red blood cell* or RBC*) adj5 (transfus* or infus* or unit* or therap*)).ti,ab.
25. ((red cell* or RBC* or erythrocyte* or red blood cell* or whole blood or transfus*) adj5 (use* or usage* or utiliz* or utilis* or requir* or need* or administ* or replac* or support* or strateg* or management or practic* or indicat* or criteri* or standard* or program*)).ab.
26. ((red cell* or RBC* or erythrocyte* or blood or transfus*) and (use* or usage* or utiliz* or utilis* or requir* or need* or administ* or replac* or support* or strateg* or management or practic* or indicat* or criteri* or standard* or program*)).ti.
27. (allogeneic blood or (unit* adj2 blood) or allogenic blood or (blood adj2 exposure) or donor blood or blood product* or blood component* or blood support).ti,ab.
28. (hemotransfus* or haemotransfus* or hypertransfus* or hemotherap* or haemotherap*).tw.
29. (red cell* or erythrocyte* or blood or RBC*).tw. and transfus*.ti.
30. 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29
31. BLOOD COMPONENT THERAPY/
32. GRANULOCYTE TRANSFUSION/ or LEUKOCYTE TRANSFUSION/ or LYMPHOCYTE TRANSFUSION/ or PLASMA TRANSFUSION/ or THROMBOCYTE TRANSFUSION/
33. 31 not 32
34. ERYTHROCYTE/
35. (red cell* or red blood cell* or erythrocyte* or RBC*).tw.
36. 34 or 35
37. 33 and 36
38. 30 or 37
39. *HEMATOCRIT/
40. ((h?emoglobin or h?ematocrit* or HB or HCT) adj5 (level* or concentration* or target* or maintain* or rais* or higher or lower or greater or above or below or equal or transfus*)).tw.
41. 39 or 40
42. 38 and 41
43. ((transfus* or red cell* or red blood cell* or RBC*) adj10 (trigger* or thresh?old* or target* or restrict* or liberal* or aggressive* or conservative* or prophylactic* or limit* or protocol* or policy or policies or practice* or standard*)).tw.
44. (((transfus* or red cell* or red blood cell* or RBC* or h?ematocrit*) and (level* or critical* or intensive* or h?emorrhag* or bleed*)) or hypertransfus*).ti.
45. 42 or 43 or 44
46. 21 and 45

CINAHL (EBSCOHost)

S1	(MH "Blood Transfusion")
S2	(MH "Erythrocyte Transfusion")
S3	(erythrocyte* or red cell* or red blood cell* or blood or RBC*) N5 (transfus* or infus* or therap* or unit*)
S4	AB ((red cell* or RBC* or erythrocyte* or red blood cell* or whole blood or transfus*) N5 (use* or usage* or utiliz* or utilis* or requir* or need* or administ* or replac* or support* or strateg* or management or practic* or indicat* or criteri* or standard* or program*))

S5 TI ((red cell* or RBC* or erythrocyte* or blood or transfus*) and (use* or usage* or utiliz* or utilis* or requir* or need* or administ* or replac* or support* or strateg* or management or practic* or indicat* or criteri* or standard* or program*))
S6 hemotransfus* or haemotransfus* or hemotherap* or haemotherap* or hypertransfus*
S7 TX (red cell* or erythrocyte* or blood or RBC*) and TI (transfus*)
S8 S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7
S9 (MM "Hematocrit")
S10 TX ((hemoglobin or haemoglobin or hematocrit* or haematocrit* or HB or HCT) N5 (level* or concentration* or target* or maintain* or rais* or higher or lower or greater or above or below or equal or transfus*))
S11 S9 OR S10
S12 S8 AND S11
S13 TI ((transfus* or red cell* or red blood cell* or RBC* or haematocrit* or hematocrit*) and (level* or critical* or intensive* or haemorrhag* or hemorrhag* or bleed*))
S14 TX ((transfus* or "red cell*" or "red blood cell*" or RBC*) N10 (trigger* or threshold* or target* or restrict* or liberal* or aggressive* or conservative* or prophylactic* or limit* or protocol* or policy or policies or practice* or standard*))
S15 S12 OR S13 OR S14
S16 (MH Clinical Trials+)
S17 PT Clinical Trial
S18 TI ((controlled trial*) or (clinical trial*)) OR AB ((controlled trial*) or (clinical trial*))
S19 TI ((singl* blind*) OR (doubl* blind*) OR (trebl* blind*) OR (tripl* blind*) OR (singl* mask*) OR (doubl* mask*) OR (tripl* mask*)) OR AB ((singl* blind*) OR (doubl* blind*) OR (trebl* blind*) OR (tripl* blind*) OR (singl* mask*) OR (doubl* mask*) OR (tripl* mask*))
S20 TI randomi* OR AB randomi*
S21 MH RANDOM ASSIGNMENT
S22 TI ((phase three) or (phase III) or (phase three)) or AB ((phase three) or (phase III) or (phase three))
S23 (TI (random* N2 (assign* or allocat*))) OR (AB (random* N2 (assign* or allocat*)))
S24 MH PLACEBOS
S25 S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24
S26 S12 AND S25

PUBMED (epublications only)
#1 ("erythrocyte transfusion*" OR "red cell transfusion" OR "red cells" OR "red blood cells" OR "red blood cell transfusion" OR "blood transfusion*" OR RBCs OR "RBC transfusion*" OR hemotransfus* OR haemotransfus* OR hemotherap* OR haemotherap* OR hypertransfus*)
#2 ((hemoglobin OR haemoglobin OR haematocrit* OR hematocrit* OR HB OR HCT) AND (level* or concentration* OR target* OR maintain* OR rais* OR higher OR lower OR greater OR above or below OR equal OR transfus*))
#3 (random* OR blind* OR control group* OR placebo OR controlled trial OR controlled study OR trials OR systematic review OR meta-analysis OR metaanalysis OR literature OR medline OR cochrane OR embase) AND ((publisher[sb] OR inprocess[sb]) NOT pubstatusnihms)
#4 #1 AND #2 AND #3

LILACS
((tw:(transfused OR transfusing OR transfused OR hypertransfusion OR haemoglobin OR hemoglobin OR haematocrit OR hematocrit) AND (instance:"regional") AND (db:("LILACS") AND type of_study:("clinical_trials"))) AND (instance:"regional") AND (year_cluster:("2009" OR "2010" OR "2011" OR "2012" OR "2013" OR "2014"))

TRANSFUSION EVIDENCE LIBRARY
Subject Area: Blood Components/Red Cells
OR
All fields: trigger OR triggers OR threshold OR thresholds OR haemoglobin OR haemoglobin OR haematocrit OR hematocrit OR Hb OR HCT

WEB OF SCIENCE – CPCIS database
#1 TS=(("erythrocyte transfusion*" OR "red cell transfusion" OR "red cells" OR "red blood cells" OR "red blood cell transfusion" OR "blood transfusion*" OR RBCs OR "RBC transfusion*" OR hemotransfus* or haemotransfus* or hemotherap* or haemotherap* or hypertransfus*)

#2 TS=((hemoglobin or haemoglobin or hematocrit* or haematocrit* or HB or HCT) NEAR/1 (level* or concentration* or target* or maintain* or rais* or higher or lower or greater or above or below or equal or transfus*))
#3 #1 AND #2
#4 TS=((transfus* or "red cell*" or "red blood cell*" or RBC*) NEAR/5 (trigger* or threshold* or target* or restrict* or liberal* or aggressive* or conservative* or prophylactic* or limit* or protocol* or policy or policies or practice* or standard*))
#5 #3 OR #4
#6 TS=(randomi* OR randomly OR "random assignment" OR "random allocation" OR blind* OR "control group*" OR controlled trial OR "controlled study")
#7 #5 AND #6

Ongoing Trials:

ClinicalTrials.gov & WHO ICTRP

Search Terms/Title: randomized OR randomised OR randomly
Intervention: red cell transfusion OR RBC transfusion OR blood transfusion
Title: trigger OR threshold OR target OR restrictive OR liberal OR aggressive OR conservative OR prophylactic OR limit OR protocol OR policy OR policies OR practice OR standard OR hemoglobin OR hematocrit

ISRCTN & EUCTR [terms searched in combination and individually]

(red cell transfusion OR red blood cell transfusion OR RBC transfusion OR blood transfusion) AND (trigger OR threshold OR target OR restrictive OR liberal OR aggressive OR conservative OR prophylactic OR limit OR protocol OR policy OR policies OR practice OR standard OR hemoglobin OR hematocrit OR haemoglobin OR haematocrit)

Hong Kong Clinical Trials Registry [terms searched individually]

Disease Group: Blood and blood-forming organs OR Circulatory System

Title: randomized OR randomised OR transfusion

4. Early Troponin I in critical illness and its association with hospital mortality: a cohort study

We have linked TnI taken within 24 hours of ICU admission to hospital mortality and Scottish Intensive Care Society Audit Group data, to investigate the association between TnI and hospital mortality in the general ICU population. This has been accepted by Critical Care (in press), and has been included in its accepted form in line with Edinburgh University guidelines.

Early Troponin I in critical illness and its association with hospital mortality: a cohort study

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Total word count: 3,640

Running Title: Early Troponin I in ICU and hospital mortality

Abstract

Background: Troponin I (TnI) is frequently elevated in critical illness, but its interpretation is unclear. Our primary objectives were to evaluate whether TnI is associated with hospital mortality, and if this association persists after adjusting for potential confounders. We also aimed to ascertain whether addition of TnI to the APACHE II risk prediction model improves its performance in general ICU populations.

Methods: We performed an observational cohort study, with independent derivation and validation cohorts in two general level 3 ICU departments in the UK. Derivation cohort: 4.5 year cohort (2010-2014) of general ICU index admissions (n=1,349). Validation cohort: Secondary analysis of prospective study dataset (2010) (n=145). The primary exposure was plasma TnI concentration taken within 24 hours of ICU admission. The primary outcome was hospital mortality. We performed multivariable regression adjusting for components of the APACHE II model. We derived the risk prediction score from the multivariable model with TnI.

Results: Hospital mortality was 37.3% (n=242) for patients with detectable TnI, compared with 14.6% (n=102) for patients without detectable TnI. There was a significant univariable association between TnI and hospital mortality (OR per doubling TnI 1.16, 95%CI 1.13, 1.20, $p<0.001$). This persisted after adjustment for APACHE II model components (OR TnI 1.05, 95%CI 1.01, 1.09, $p=0.003$). TnI correlated most strongly with the Acute Physiological Score (APS) component of APACHE II ($r=0.39$). Addition of TnI to the APACHE II model did not improve discrimination (APACHE II c-index 0.835 (95%CI 0.811, 0.858); APACHE II+TnI c-index 0.837 (95%CI 0.813, 0.860), $p=0.330$), or improve other measures of model performance.

Conclusions: TnI is an independent predictor of hospital mortality, and correlates most highly with the APS component of APACHE II. It does not improve risk prediction. We would not advocate the adoption of routine troponin analysis on admission to ICU, and recommend that troponin is measured only if clinically indicated.

Word count: 305

Key words: troponin, critical care, hospital mortality

INTRODUCTION

Troponin is used routinely in combination with clinical signs, symptoms, and electrocardiography, to diagnose or rule out myocardial infarction (MI)(80), and as a prognostic marker in pulmonary embolism(158). In critical illness, Troponin is frequently elevated but its interpretation in this context is uncertain. Several studies in intensive care unit (ICU) and high risk surgical populations describe significant crude associations between troponin elevation and increased hospital and longer-term mortality (95, 97, 159). There has been increasing interest in the use of troponin in risk stratification in major non-cardiac surgery: the VISION study recently found that peak postoperative troponin T was significantly associated with 30 day mortality, after adjustment for patient characteristics, and type of surgery(160). However, the mechanism of a causal association between troponin elevation and mortality is uncertain. Specifically, the aetiology of troponin release is likely multifactorial and the relative importance of inflammatory and ischaemic myocardial injury are uncertain. In addition, there is conflicting evidence whether troponin is an independent predictor of hospital mortality after adjustment or stratification for severity of illness in critically ill patients(82, 161-163).

The use of multivariable risk prediction models which include diagnosis, severity of illness and other patient characteristics is standard in intensive care and enables benchmarking of practice and outcomes across units(164). The Scottish Intensive Care Society Audit Group (SICSAG) uses the APACHE II model (Acute Physiological and Chronic Health Evaluation II)(165), which combines acute physiological derangement and presenting diagnosis with chronic health status and age. It has been validated and recalibrated using Scottish data(166). Addition of new variables may improve the accuracy of existing models; for example the UK Intensive Care National Audit and Research Centre (ICNARC) has recently improved the accuracy and discrimination of its model by including lactate(167). The strong univariable association of troponin with mortality makes it an ideal candidate predictor variable to assess in current risk prediction models.

We aimed to explore the possible independent relationship between early Troponin I (TnI) and hospital mortality in ICU patients. We explored univariable associations between early TnI concentration and mortality. We then explored how this relationship was modified by adjustment for potential confounding variables that predict death during the first 24 hours of ICU admission. Finally, we investigated whether the addition of TnI to the existing APACHE II risk prediction model improved its performance as a predictor of hospital mortality in general critical care populations.

METHODS

Data sources and participants

Two prospectively collected datasets were available with routine TnI measurement in ICU patients from two distinct ICUs. Glasgow Royal Infirmary (GRI), Scotland, collected TnI samples on all patients admitted to the ICU three times each week (Mondays, Wednesdays and Fridays) from 01/01/2010-30/06/2014. These measurements were supplemented by clinically indicated TnI measurements at clinician discretion (Glasgow dataset). Ostermann et al also performed routine TnI (in addition to their published TnT) within 24 hours of ICU admission to a large London ICU (St Thomas' hospital) for all patients recruited to an observational study that investigated the impact of serial troponin measurements on the diagnosis of myocardial infarction and hospital and six-month mortality in patients admitted to ICU with non-cardiac diagnoses (London dataset) (95).

For the Glasgow dataset we linked TnI data, clinical data extracted from the hospital clinical information system (CareVue®), and routinely collected administrative linked registry data derived from the Scottish Intensive Care Society Audit Group (SICSAG) (168), Scottish Morbidity Record of acute hospital admissions (SMR01), and Scottish death records. All data were anonymised prior to release to the researchers, and therefore an ethics waiver was granted by the local research ethics committee (West of Scotland Research Ethics Service), and approvals from NHS Greater Glasgow and Clyde Caldicott Guardian, and SICSAG were obtained.

Methods for the London dataset have previously been described elsewhere (95). TnI measurements were part of the original protocol and were reported in a subsequent publication (169). Data were anonymised prior to release to the researchers. We restricted the dataset to patients in whom TnI measurements were taken in the first 24 hours of ICU admission. All diagnoses were recorded as free text, which we mapped to APACHE II diagnosis categories.

Variables

The primary outcome was hospital mortality. The primary predictor was TnI. We entered TnI as a continuous variable. We assessed linearity of TnI using fractional polynomials and identified that a logarithmic transformation of TnI best represented the data. We used a base 2 logarithmic transformation to facilitate clinical interpretation.

Plasma TnI for the Glasgow dataset was measured using the Abbott ARCHITECT STAT troponin I assay (limit of detection 0.01mcg/l, coefficient of variation (CoV) 10% at 0.04mcg/l, quoted analytical range 0.00 to 50.00mcg/l); the assay for the London dataset was the Siemens TnI-Ultra three-site sandwich immunoassay on the Advia Centaur. The quoted analytical range was 0.006-50mcg/l, total CoV were 2.7-5.3% (measured between 0.08-27.2mcg/l), reference range<0.039mcg/l. The lower limit for reporting was 0.04mcg/l. The assays remained the same throughout the study periods. We classified TnI<0.04mcg/l (undetectable) as "0mcg/l", and therefore added a small constant (log2 (TnI + 0.001)) to all values. We described the study cohort stratified by TnI status for ease of clinical interpretation.

Association of TnI and hospital mortality

We performed univariable logistic regression to assess the unadjusted association of TnI and hospital mortality, and multivariable logistic regression to assess the association of TnI and hospital mortality after adjustment for confounders. For multivariable analyses, we adjusted for the APACHE II risk prediction model. In order to explore potential mechanisms underlying the association between TnI and hospital mortality, we assessed individual components of the APACHE II risk prediction model: age, chronic health points, emergency surgical admission, Acute Physiology Score and APACHE II diagnosis on ICU admission. We assessed correlation between TnI and components of the APACHE II model using the square root of the R^2 value (see online supplement). We then adjusted for each component separately for the relationship between hospital mortality and TnI, and compared the impact that each component had on the odds ratio for hospital mortality by TnI. For details regarding missing data, see online supplement.

We performed a number of subgroup and sensitivity analyses for the associations between TnI and hospital mortality

i) Routine versus clinical TnI collection: The Glasgow dataset comprised all TnI samples taken within 24 hours of ICU admission. We compared characteristics and outcomes of patients who had a TnI sample taken with routine morning bloods on a Monday/Wednesday/Friday ("Routine"), with those who had a TnI on Tuesday/Thursday/Saturday/Sunday at clinical discretion ("Clinical"). We also restricted analyses to patients who had routine TnI samples taken, to explore any bias that might result from inclusion of clinically indicated TnI samples.
ii) We restricted analyses to patients with an APACHE II comorbidity diagnosis of severe cardiac disease (New York Heart Association NYHA 4). We hypothesised the mechanism of TnI release may be different in patients with severe cardiovascular disease.
iii) We included an interaction term for sex because TnI release is higher in men than women, due to increased muscle mass of the left ventricle(170).
iv) We entered TnI as a binary categorical variable consistent with diagnostic thresholds for this assay: "Positive" (above the limit of reporting ≥0.04) vs "Negative" (below the limit of reporting, <0.04).

Sensitivity analysis

A significant proportion of patients did not have TnI taken within 24 hours of admission. For those patients who had samples taken at clinical discretion, we believe that these are "missing not at random", as the mechanism of missingness is likely related to the TnI value. However, for those samples taken as part of routine care, we believe that missing values may fulfil criteria to be "missing at random." We therefore imputed the missing TnI values using multiple imputation by chained equations to assess the robustness of our analysis for this subgroup of patients (online supplement).

Addition of TnI to the APACHE II risk prediction model

To explore whether TnI concentration measured during the first 24 hours in the ICU added to the risk prediction properties of the APACHE II, we undertook a derivation and validation of the model using the datasets with and without the inclusion of TnI data.

For model development, we reconstructed the APACHE II risk prediction model (165) using raw data and derived the predicted mortality empirically from the multivariable model. We constructed age points, chronic health points, emergency surgical admission and Acute Physiological Score in the same way as the APACHE II risk prediction model. We modified the APACHE diagnostic codes by recoding diagnostic codes with frequency <10 into "other" disorder for that system to ensure stability of coefficient estimates. We then added TnI to the APACHE II model, using empirically weighted coefficients derived from the model. This ensured that we did not underestimate any potential contribution to the model from TnI (14].

For model validation, we used the whole Glasgow dataset to develop the model, and applied coefficients from the development dataset to the London dataset in line with current guidelines (171).

We reported the performance of the risk prediction model for the following: calibration, discrimination, area under the receiver operating characteristic curve (c-index, DeLong's test for two correlated roc curves), overall performance score (Brier score), and overall fit (Akaike information criterion (AIC) and R^2) (171).

We compared how the performance of the APACHE II risk prediction model was altered by the inclusion of TnI on the first day in ICU as evidence for early contribution to risk of death.

All data were analysed using R (R Core Team v3.3.2, Vienna, Austria) (172). We used the following packages: ggplot2, mfp, rms, pROC (173-176) (R code: Online supplement).

RESULTS:

Participants

There were 3,073 index admissions to GRI between 01/01/2010 and 30/06/2014 (Figure 4). 1,349 (43.9%) patients had TnI taken within 24 hours of admission to ICU ("Glasgow dataset"). Patients who did not have TnI taken (n=1724, 56.1%) were younger, were more likely to be an elective admission, and had lower APACHE II scores and mortality (ICU, hospital and 6 month) (Table E1). The mean age of patients who had TnI taken within 24 hours was 59.6 years (SD 16.6), 607 (45.0%) were female and 59.5% were emergency medical admissions (Table 6). 80.0% had no severe comorbidity (APACHE II), the mean APACHE II score was 20.1 (8.1) and median predicted mortality was 31.7%. Patients with positive (TnI≥0.04mcg/l) compared with negative (TnI<0.04mcg/l) TnI were older, and a higher proportion were male, emergency medical admissions, with higher APACHE II scores, and mortality (ICU, hospital, 6 month) (Table 6). Median TnI in those with a detectable level (n=648) was 0.23mcg/l (Q1 0.09, Q3 0.97, max 69.91).

Association of TnI and hospital mortality

Hospital mortality was 37.3% (n=242) for TnI positive patients, compared with 14.6% (n=102) for TnI negative patients (Table 6). There was a significant univariable association between TnI as a continuous term and hospital mortality (Figure 5A), odds ratio per doubling of TnI 1.16 (95% CI 1.13, 1.20, p<0.001). This is equivalent to an increase in predicted hospital mortality from 27.2% to 30.3% if TnI increases from 0.04mcg/l to 0.08mcg/l; and from 42.9% to 46.7% for an increase in TnI from 1.0mcg/l to 2.0mcg/l. After adjustment for the APACHE II risk prediction model, TnI remained an independent predictor of hospital mortality, but the magnitude of prediction was reduced (Figure 5B) (OR 1.05, 95% CI 1.01 to 1.09, p=0.003).

Correlation of TnI with components of the APACHE II model

TnI was most highly correlated with the Acute Physiological Score (r=0.39) and diagnostic category (r=0.40) components of the APACHE II model (Table 2). There was some correlation with age points (r=0.17), but minimal correlation with chronic health points (r=0.11) or emergency surgery (r=0.10). This was reflected by the impact that each component had on the association between TnI and hospital mortality. APS had the greatest impact, reducing the odds of hospital mortality from 1.16 (95% CI 1.13 to 1.20) to 1.08 (95% CI 1.05 to 1.11).

Addition of TnI to the APACHE II risk prediction model

Derivation: The variables comprising the APACHE II model applied to this dataset resulted in a c-index of 0.835 (95% CI 0.811 to 0.858, Figure 6) demonstrating good discriminatory ability and calibration (Figure E1). The addition of TnI as a logarithmic term did not improve the discriminatory power of the model (p=0.330; c-index 0.837, 95% CI 0.813 to 0.860, Figure 3) nor improve other assessments of model performance (**TABLE 8**, Figure E2).

Validation: There were 145 patients in the London dataset, with similar demographics to the Glasgow dataset (Table 6, Table E3). There was a high proportion of emergency medical admissions (71.0%), with a mean APACHE II score 19.1. Hospital mortality was 20.0%. TnI was significantly associated with hospital mortality (unadjusted OR TnI 1.23, 95% CI 1.09 to 1.41). This association was attenuated after adjustment for potential confounders (OR 1.16 (95% CI 0.99 to 1.36)).

Applying coefficients derived from the Glasgow data derived model, we found a small but statistically significant improvement in the c-index after the addition of TnI; however confidence intervals were wide (standard APACHE II model c-index 0.735 (95% CI 0.631 to 0.845), APACHE II + TnI c-index 0.752 (95% CI 0.645 to 0.859) p=0.010). Other assessments of model performance showed no improvement with the addition of TnI (**TABLE 8**).

Subgroup and sensitivity analyses: Multivariable association of TnI and hospital mortality

Those with a routine TnI sample accounted for 55.3% of samples taken (n=746). Those with a TnI sample taken at clinical discretion accounted for 44.7% (n=603) (Table E1). "Clinical" group patients were more likely to be emergency admissions, have a positive TnI, have higher APACHE II scores and predicted mortality. ICU length of stay, ICU mortality, and hospital or six months mortality were similar. Restriction to "Routine" group patients did not alter the magnitude of association of TnI in the multivariable model but the association was no longer statistically significant (OR 1.03, 95%CI 0.98 to 1.09, p=0.287, Figure 7). The OR for TnI in our sensitivity analysis where we imputed the missing TnI values in the "Routine" group was 1.04 (95% CI 1.00 to 1.08, p=0.020, Figure 5, Table E2).

TnI was a significant predictor of hospital mortality when the dataset was restricted to patients (n=49) with a diagnosis of severe cardiac disease disease (OR 1.20, 95%CI 1.00 to 1.49, Figure 7).

The odds of hospital mortality were greater for men (OR 1.08, 95%CI 1.03 to 1.13) than women (OR 1.03, 95%CI 0.98 to 1.08, Figure 7), but this difference was not significant when tested for interaction between TnI and sex (p=0.181, Figure E2).

When TnI was entered as a binary variable "Positive" (n=648) vs "Negative" (n=701), the odds of hospital mortality for positive TnI was 1.43 (95% CI 1.03 to 1.99) (Figure 7).

DISCUSSION

In this cohort study of two independent datasets, we found that an early raised TnI level was associated with increased mortality in general ICU populations. The magnitude of the association remained significant but was markedly attenuated after controlling for other important predictors of hospital mortality. The addition of TnI to the APACHE II risk prediction model did not result in a clinically relevant improvement of model performance.

Our study has a number of strengths. We had access to routinely measured TnI in a large well-defined population in the derivation cohort, and a high quality validation cohort from a prospective study in a different population and setting. This contrasts with previous ICU studies which have analysed TnI samples taken for clinical purposes (82, 161). This reduced the risk of bias by clinical indication. Although there have been several large peri-operative studies, this is the largest study to date to investigate troponin in ICU patients, allowing subgroup analyses to assess the robustness of our findings. We used multiple imputation for missing TnI values in patients who were eligible for routine TnI sampling. We also used TnI as both a continuous and a categorical variable. We reported our risk prediction model to current international best practice standards, including externally validating the model rather than internally validating on a proportion of the derivation cohort (171). Internally validated prediction models only make use of data in the development model, and may have an artificially high performance. The external validation dataset provided the heterogeneity that will be encountered in the real-life application of the model (167).

Our study has some limitations. Patients who did not have TnI taken within 24 hours of admission to ICU had differences from those who did, which may affect the generalisability of findings. This could have occurred because ICU case mix can differ between certain weekdays and weekends (177, 178), and because patients who were less sick did not have blood samples taken. Patients who were eligible for routine TnI sampling, but did not receive it were younger, less sick, and had much shorter ICU stays. Troponin was reported using the assays in clinical practice during the study. The assays used in the Glasgow and London studies were different which may have affected interpretation of the London dataset. Our lower limit of reporting was 0.04mcg/l, which may have resulted in some samples being classified as "0"mcg/l, when TnI may have been detectable using a highly sensitive assay, and this may have affected women more (170). We found no significant interaction between TnI and sex, although our study may be underpowered for this.

A further limitation was the restriction of troponin data to the first 24 hours of ICU care. This time point is defined by hospital location rather than illness stage, and is therefore potentially subject to lead time bias. For troponin this could be particularly relevant, given the important association between time of symptom onset and troponin measurement for maximum diagnostic value in acute coronary syndrome. This cannot be controlled in ICU populations, but was a potential source of variation. We restricted our analyses to the first TnI taken, but assessing the dynamics of TnI release during critical illness may have greater prognostic value. Future research could explore the dynamics of troponin release in critically ill patients, which might have a stronger association with mortality.

The significant univariable association between TnI and hospital mortality confirms that found in other studies undertaken in critically ill and high risk surgical populations (95, 97, 159, 161-163, 179). In non-cardiac surgical patients, troponin T (TnT) has also been found to have a significant independent association with hospital and

longer term mortality (97, 179). Both studies made adjustment for patient characteristics, and type of surgery, but did not include any measure of acute physiological derangement. In critically ill patients, Wu et al found a significant association between TnI and six month mortality after stratifying by high/low APACHE II scores (162). Babuin et al. found that TnT remained a significant predictor of mortality after adjustment for APACHE III (161). This was a retrospective study of patients with a high proportion of cardiac disease who had TnT taken for clinical indications. This is consistent with our finding that, in patients with chronic heart disease, TnI remained a predictor of mortality after adjustment.

Our finding that the association between TnI and mortality was significant but markedly attenuated after adjusting for potential confounders suggests that myocardial injury may be on the causal pathway between illness severity and death: the sicker the patient, the more troponin is released, and the higher the risk of death. The causal mechanism of TnI release is unclear. Troponin release has traditionally been associated with myocardial necrosis and forms a key part of the diagnosis of myocardial infarction (80). However, there is also increasing evidence that troponin release may occur during myocardial ischaemia, in the absence of myocardial necrosis, potentially due to shedding of cytosolic troponin (180). The physiological stress associated with critical illness may exacerbate oxygen supply/demand imbalance (Type II MI) (80, 181), particularly in those with underlying critical coronary artery disease. In sepsis, there are profound haemodynamic alterations in the microcirculation which may lead to alterations in oxygen extraction and tissue oxygenation (182). In a minority of critically ill patients, troponin release may be caused by increased thrombogenicity leading to coronary plaque rupture and thrombosis (Type I MI). Proposed non-cardiac mechanisms of troponin release in the critically ill include direct toxicity from cytokine release such as TNF-alpha and interleukin-6 (183), stretch mediated troponin release (183), or ongoing subclinical myocardial injury due to uraemia and impaired excretion (83). Our datasets did not allow us to explore in detail underlying mechanisms of troponin release. However, the APS component represents acute physiological stress, and comprises surrogate markers of supply (hypoxia, hypotension, anaemia) versus demand (increased metabolic rate) imbalance, and our finding that TnI is most strongly correlated with the APS component of the APACHE II model supports the hypothesis that myocardial injury may be due to oxygen supply-demand imbalance. Using Bradford-Hill's criteria for assessing causal relationships, we showed that there was a biological gradient, where a greater TnI was associated with increased mortality, and that there was consistency across the literature. However, there was no TnI measurement preceding critical illness, meaning that we were unable to comment on the temporal association. Furthermore, the strength of association between TnI and mortality after adjustment for known confounders was weak, and the mechanism of the association was unclear. This was an observational study, and unmeasured confounders prevent the inference of causality. Further exploration would require propensity or mediation analyses.

CONCLUSION

In conclusion, we found that the significant association between TnI and hospital mortality was substantially attenuated after controlling for potential confounders, particularly acute physiological derangement. However, the addition of TnI to the existing APACHE II model did not improve its performance. Given its relative expense we would not advocate the adoption of routine troponin analysis for general ICU patients on their admission to critical care, and would recommend that troponin is measured only if clinically indicated. Future

studies using higher generation highly sensitive troponin assays, potentially exploring changes over time, may further inform the relationship between TnI and mortality.

DECLARATIONS

List of Abbreviations

TnI: Troponin I

MI: Myocardial Infarction

ICU: Intensive Care Unit

APACHE II: Acute Physiological and Chronic Health Evaluation

Scottish Intensive Care Society Audit Group: SICSAG

Scottish Morbidity Record of acute hospital admissions: SMR01

Ethics approval

All data were anonymised prior to release to the researchers, and therefore an ethics waiver was granted by the local research ethics committee (West of Scotland Research Ethics Service, 06/09/15), and approvals from NHS Greater Glasgow and Clyde Caldicott Guardian (04/06/15), and SICSAG were obtained (06/05/15).

Consent for publication

All data were anonymised prior to release to the researchers, and therefore an ethics waiver for consent was granted by the local research ethics committee

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

Nil

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Authors' Contributions

ABD, MS, JK, TSW and NL conceived the study. AD, MS, MO, MA and JO performed the data collection and data linkage. AD and NL performed the data analysis. All authors contributed to the data interpretation and writing. All authors read and approved the final manuscript.

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We would also like to thank Dr David Treacher who led the prospective troponin study in London and approved the collaboration with the team in Edinburgh, and the research nurses K Lei, J Smith and B Sanderson for collecting the troponin samples.

Additional files:

Online supplement_TnI Critical Illness_revised.doc
Online supplementary material

Table 6: Baseline characteristics. Stratified by Overall population, TnI –ve (<0.04mcg/l) vs TnI +ve (≥0.04mcg/l). P-value: test between TnI –ve and TnI +ve: Chi2 test/test for trend for categorical variables, t-test for parametric continuous variables, Mann-Whitney-U test for non-parametric variables. LOS: length of stay. There are no missing data in this table, patients with missing TnI values are discussed in the supplement.

	Overall	**%**	**TnI -ve**	**%**	**TnI +ve**	**%**	**P value**
n	1,349		701		648		
Age mean (sd)	59.6	16.6	56.7	16.8	62.8	15.8	<0.001
Female (%)	607	45.0	337	48.1	270	41.7	0.021
Admission type (%)							<0.001
Elective surgery	333	24.7	198	28.2	135	20.8	
Emergency surgery	213	15.8	172	24.5	41	6.3	
Emergency medical	803	59.5	331	47.2	472	72.8	
Deprivation quintile (%) (n=1,143)							0.081
1 (most deprived)	622	46.1	298	42.5	324	50.0	
2	193	14.3	108	15.4	85	13.1	
3	94	7.0	52	7.4	42	6.5	
4	123	9.1	66	9.4	57	8.8	
5 (least deprived)	111	8.2	68	9.7	43	6.6	
APACHE comorbidities							0.030
0	1,079	80.0	589	84.0	490	75.6	
1	191	14.2	83	11.7	109	16.8	
≥2	79	5.9	29	4.1	49	7.6	
TnI mcg/l med [IQR] max	0.0	[0.00,0.21] 69.91	-	-	0.23	[0.09,0.96] 69.91	
Outcomes							
ICU mortality (%)	292	21.6	73	10.4	219	33.8	<0.001
Hospital mortality (%)	344	25.5	102	14.6	242	37.3	<0.001
6 month mortality (%)	419	31.1	142	20.3	277	42.7	<0.001
% APACHE II predicted mortality med [IQR]	25.3	[9.7, 49.7]	17.2	[6.9, 32.2]	40.4	[19.8, 63.1]	<0.001
APACHE II score mean (sd)	20.1	8.1	16.9	6.6	23.6	8.1	<0.001
ICU los (med [IQR])	2.9	[1.3, 6.8]	2.7	[1.1, 6. 6]	3.2	[1.6, 7.7]	<0.001

TABLE 7: Correlation of APACHE II with TnI.

*Initially, each component was separately added to a univariable analysis with log2(TnI +0.001) as the dependent variable. The Pearson coefficient (r) is the square root of the R^2 (Coefficient of Determination), and assesses the correlation between the two variables. *We adjusted for each component separately for the relationship between hospital mortality and TnI, and compared the impact that each component had on the odds ratio for hospital mortality by TnI. Unadjusted OR per doubling TnI: 1.16 (95% CI 1.13, 1.20), p<0.001.*

Component of APACHE II	**Pearson Coefficient (r)**	**TnI Odds Ratio for hospital mortality***	**95% CI**
Unadjusted TnI	-	*1.16*	*1.13, 1.20*
Age points	0.17	1.15	1.12, 1.19
Chronic health points and Emergency Surgery	0.11	1.16	1.13, 1.19
APS	0.39	1.08	1.05, 1.11
Emergency surgery	0.10	1.16	1.12, 1.19
Diagnostic category	0.40	1.11	1.07, 1.15

TABLE 8: Model Comparison for Glasgow and London datasets (derivation)

APACHE II vs APACHE II + TnI. AUC: Area under curve (Concordance Index), AIC: Akaike Information Criterion, p value: DeLong's test for two correlated ROC curves. Glasgow model coefficients applied to London dataset (validation)

	GLASGOW			**LONDON**	
	APACHE	**APACHE + TROPONIN**		**APACHE**	**APACHE + TROPONIN**
AUC (95% CI)	0.823 (0.811 to 0.858)	0.826 (0.813 to 0.860)		0.737 (0.630 to 0.845)	0.752 (0.645 to 0.859)
AIC	1198.0	1188.8		144.5	143.0
R^2	0.340	0.349		0.330	0.355
Brier score	0.141	0.140		0.120	0.118
P value		0.331			0.010

Figures

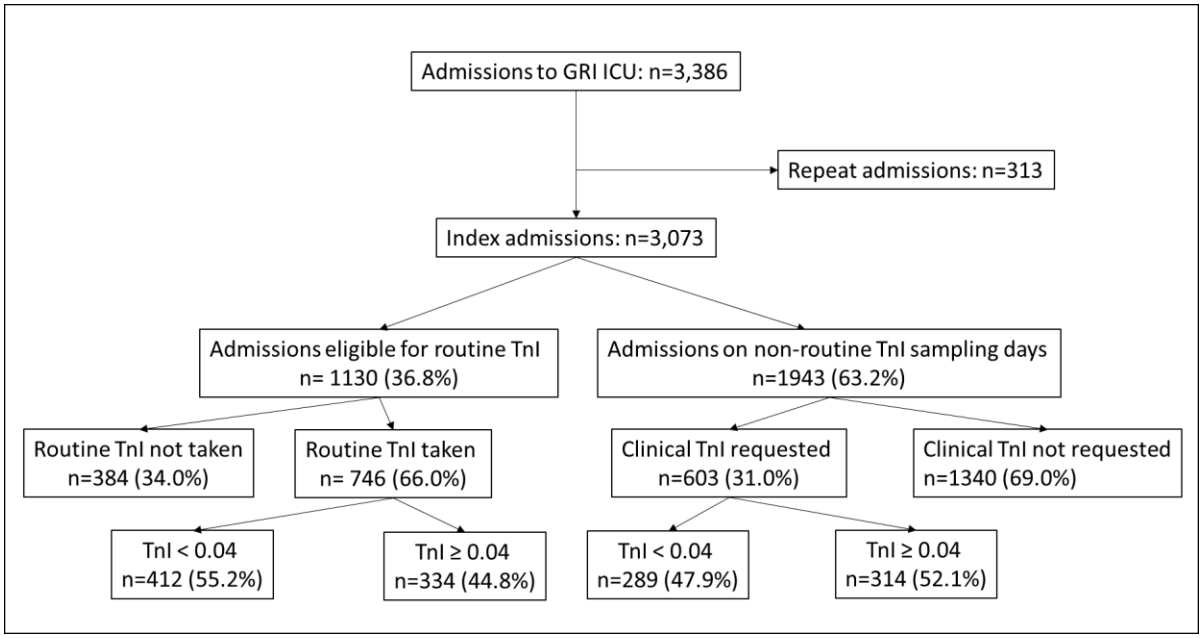

Figure 4: flow of patients through study

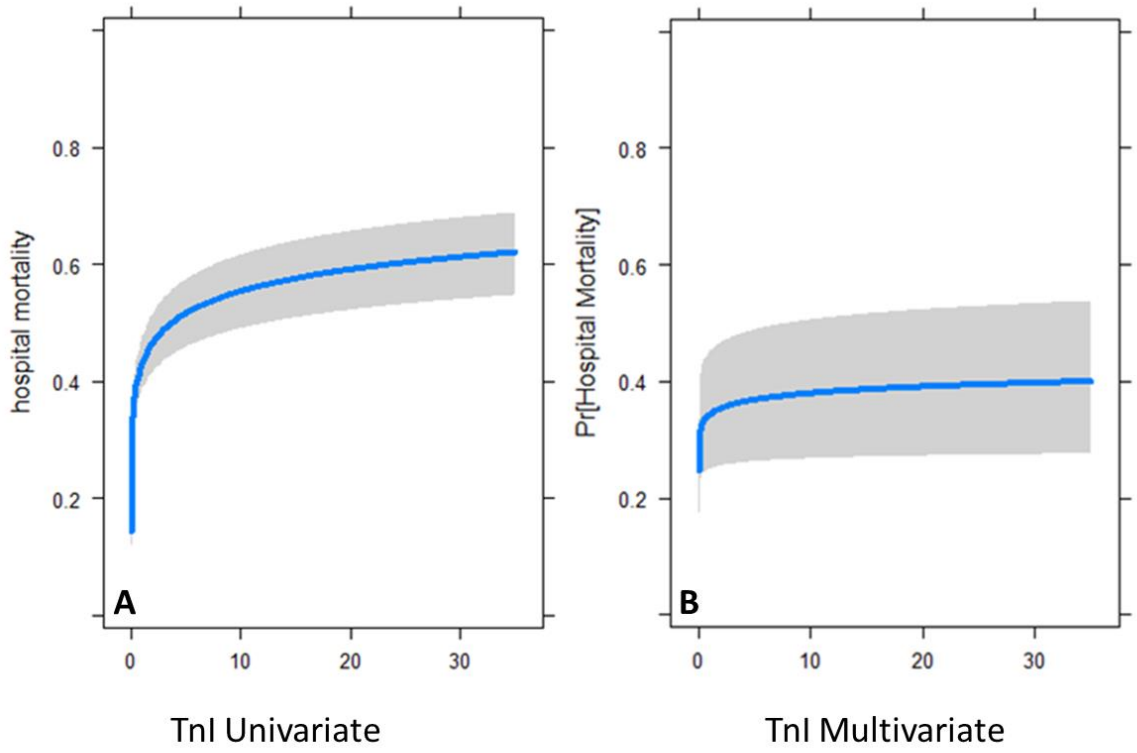

Figure 5: Association between Troponin I (mcg/l) and hospital mortality. A: Univariable association, Odds Ratio per doubling of TnI 1.16 (95% CI 1.13, 1.20), p<0.001, B: Multivariable association between TnI (mcg/l) and hospital mortality once added to the APACHE II model. OR 1.05 (95% CI 1.01, 1.09, p<0.001)

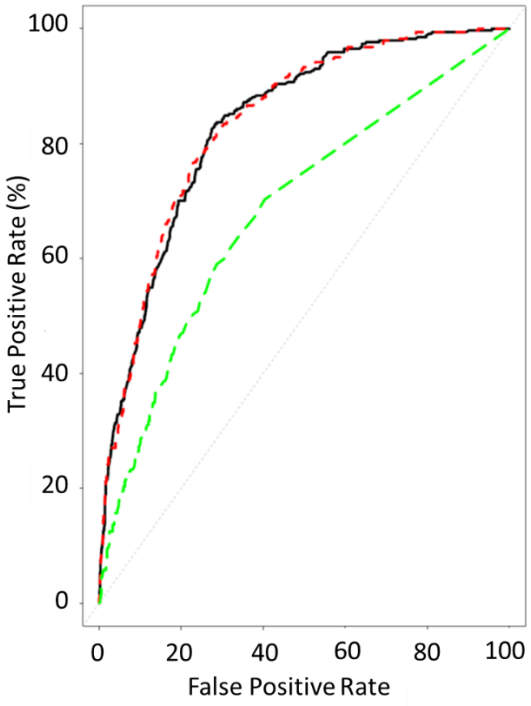

Figure 6: Receiver Operator Characteristic Curves: APACHE (black, c-index 0.847), APACHE+TN (red, c-index 0.846), TnI (green, c-index 0.696)

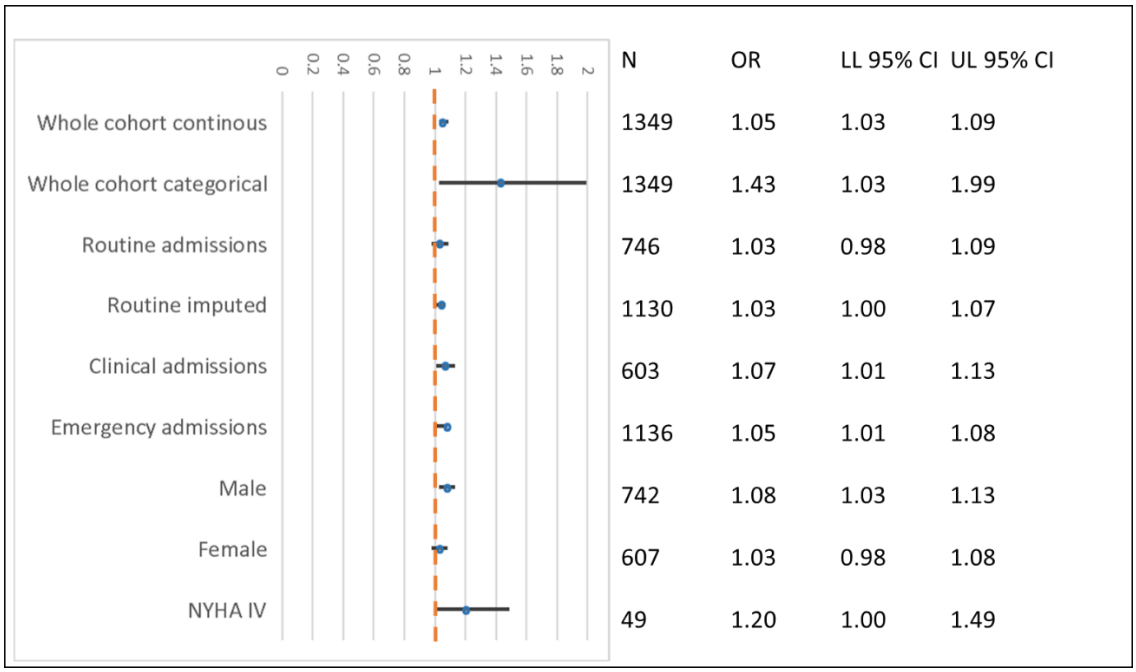

Figure 7: Sensitivity and subgroup analyses: Odds Ratio of hospital mortality for doubling of TnI as a continuous variable for different subgroups after adjusting for the APACHE II risk prediction model. 'Whole cohort categorical' refers to TnI entered as a binary variable: TnI +ve vs TnI -ve using the threshold of the limits of detection (0.04). OR Odds Ratio; LL Lower Limit; UL Upper Limit 95% Confidence Interval. NYHA: New York Heart Association Cardiac disease stage IV (APACHE II classification for severe cardiac disease).

Online supplement TnI Critical Illness

Methods

Correlation between TnI and components of the APACHE II model:

We performed univariable linear regression, with log2(TnI+0·001) as the dependent variable, and each component of the APACHE II model separately as the independent variable. We took the square root of the R^2 value, equivalent to the Pearson correlation coefficient in univariable analyses. This enabled us to include categorical predictors (not possible in simple correlation) as it uses correlation between observed TnI, and TnI predicted by the regression model (the mean TnI for each group). We then adjusted for each component separately for the relationship between hospital mortality and TnI, and compared the impact that each component had on the odds ratio for hospital mortality by TnI.

Missing data

We followed the APACHE II model method which assumes that missing physiological values are normal and are therefore allocated a zero score. Missing APACHE II diagnostic codes were classified as "other miscellaneous" in accordance with current SICSAG practice. Other missing values were not imputed and a complete case analysis was undertaken.

We stratified the dataset dependent on whether samples was taken for "routine" or "clinical" indications. For those patients who had samples taken at clinical discretion, we believe that these are "missing not at random", as the mechanism of missingness is likely related to the TnI value. It is not valid to perform multiple imputation for missing values when the missingness mechanism is "missing not at random." However, for those samples taken as part of routine care, we believe that missing values may fulfil criteria to be "missing at random." We therefore imputed the missing TnI values using multiple imputation by chained equations to assess the robustness of our analysis for this subgroup of patients

Sample size

We were limited by the sample size of the existing datasets. Regular TnI collection began for the Glasgow dataset in late 2009, and we included all index admissions between 01/01/2010-30/06/2014, who had a TnI sample taken within 24 hours of admission to ICU. The London dataset comprised 145 patients recruited to their study.

Online Tables

Table E1: Baseline characteristics. Stratified by "Routine TnI": TnI taken within 24 hours of ICU admission on Mon/Wed/Fri at 08:00; "Clinical TnI": TnI taken within 24 hours of icu admission on Tues/Thurs/Sat/Sun. "No TnI": no TnI taken within 24 hours of ICU admission. TnI positive ≥0.04, TnI negative <0.04. LOS: Length of stay

	Routine TnI	**%**	**Clinical TnI**	**%**	**No TnI**	**%**
n (%)	746	24.3	603	19.6	1724	56.1
age mean (SD)	58.4	17	61.2	16	56.2	15.8
female n (%)	334	44.8	273	45.3	858	49.8
admission type						
elective surgery	135	18.1	78	12.9	440	25.5
emergency surgery	187	25.1	146	24.2	510	29.6
emergency medical	424	56.8	379	62.9	773	44.9
deprivation quintile						
1 (most deprived)	329	44.1	293	48.6	781	45.3
2	112	15.0	81	13.4	235	13.6
3	49	6.6	45	7.5	143	8.3
4	77	10.3	46	7.6	120	7.0
5 (least deprived)	64	8.6	47	7.8	144	8.4
Missing	115	15.4	91	15.1	301	17.5
APACHE comorbidity count						
0	597	80.0	482	79.9	1410	81.8
1	109	14.6	82	13.6	230	13.3
2	27	3.6	23	3.8	54	3.1
≥3	13	1.8	16	2.6	29	1.7
TnI +ve n(%)	334	44.8	314	52.1	-	-
APACHE II score mean(SD)	19.1	8.1	21.3	7.9	15.25	7.2
APACHE predicted mortality mean (SD)	29.5	24.7	34.5	36.7	21.2	20.2
ICU LOS med (IQR)	2.9	1.3, 6.8	2.9	1.3, 6.9	1.1	0.7, 2.5
ICU mortality n(%)	150	20.1	142	23.5	202	11.7
hosp mortality n(%)	179	24	165	27.4	255	14.8
6m mortality n(%)	223	29.9	196	32.5	321	18.6

Table E2: Baseline characteristics for patients eligible for routine TnI within 24 hours of ICU admission. Stratified by whether TnI was missing or taken. 19 patients in the missing group died or were discharged before 08:00am when morning bloods were taken.

Variable	**TnI missing**	**%**	**TnI taken**	**%**	**P value**
n (%)	384	34.0	746	66.0	
age (mean (sd))	55.8	17.5	58.4	16.1	0.011
Female n (%)	200	52.2	327	43.8	0.009
Admission Type n (%)					<0.001
Elective Surgery	113	29.5	189	25.4	
Emergency Surgery	123	32.0	139	18.6	
Emergency Medical	148	38.5	419	56.1	
APACHE comorbidities n (%)					0.094
0	328	85.4	593	79.4	
1	41	10.7	46	14.8	
≥2	15	3.9	260	5.8	
Outcomes					
ICU mortality n (%)	43	11.1	132	17.7	0.004
Hospital mortality n (%)	53	13.7	158	21.2	0.003
6 month mortality n (%)	62	16.2	197	26.4	<0.001
APACHE II Score (mean (sd))	14.9	7.2	19.0	7.6	<0.001
% APACHE II predicted mortality med [IQR]	9.3	4.6, 21.3	20.8	8.6, 40.8	<0.001
ICU los med [IQR]	1.0	0.7, 2.4	3.1	1.7, 6.8	<0.001
ICU LOS<24 hours n (%)	157	40.8	83	11.1	<0.001

Table E3: Baseline Characteristics: London dataset. Stratified by Overall population, TnI –ve (<0.04mg/l) vs TnI +ve (≥0.04mg/l). P-value: test between TnI –ve and TnI +ve: Chi2 test/test for trend for categorical variables, t-test for parametric continuous variables, Mann-Whitney-U test for non-parametric variables. *OR per doubling of TnI.

	n	**%**	**TnI –ve**	**%**	**TnI +ve**	**%**	**P value**
N	145		54		91		
Age years mean (SD)	61.7	17.0	57.2	18.4	64.5	15.6	0.013
Female (%)	63	43.4	19	35.2%	43	47.3	0.213
Admission type Elective Surgery Emergency Surgery Emergency Medical	 7 38 100	 4.8 26.2 70.0	 1 13 40	 1.9 24.1 74.1	 6 25 60	 6.6 27.5 65.9	0.358
Troponin +ve	116	80	-	-	-	-	-
Troponin med (IQR,max)	0.06	0.02, 0.33	-	-	0.20	0.06, 0.81	
APACHE II score mean (SD)	19.1	6.4	14.6	7.2	20.3	8.4	0.011
Outcomes							
ICU mortality	28	19.3	1	1.9	27	29.6	0.031
Hospital mortality	29	20.0	3	5.6	38	41.8	0.010
6 month mortality	44	30.3	3	5.6	41	45.1	0.017
OR TnI (95% CI) univariable*	1.23	1.09, 1.41					
OR TnI (95% CI) multivariable*	1.16	0.99, 1.36					

Table E4: Coefficients and Standard Error for whole dataset. Hospital mortality ~ log(TnI+0.001) + APACHE II Score + Emergency surgery + APACHE II Diagnosis.

Variable	**Whole dataset**	
	Coefficient	SE
Intercept	-4.29	0.26
Log(TnI+0.001)	0.02	0.01
APACHE II Score	0.16	0.01
Emergency surgery	0.43	0.23
APACHE II Diagnosis (ref Respiratory Infection)		
Asthma/Allergy	-1.07	0.85
Other Respiratory Infection	-0.08	0.28
Pulmonary oedema (non-cardiogenic)	-0.23	0.46
Aspiration/poisoning/toxic	-0.74	0.46
Other cardiovascular disorder	-0.91	0.35
Congestive heart failure	-2.30	0.90
Sepsis	0.16	0.24
Post cardiac arrest	-0.06	0.30
Cardiogenic shock	0.47	0.47
Multiple trauma	-0.49	0.58
Head trauma	-1.13	0.66
Seizure disorder	-1.61	0.44
ICH/SDH/SAH	1.19	0.43
Other neurological disorder	-0.73	0.41
Drug overdose	-2.51	0.55
Other metabolic/renal disorder	-1.48	0.35
GI bleeding	-0.93	0.50
Other gastrointestinal disorder	0.13	0.22
Chronic cardiovascular disease	-0.69	0.54
Sepsis	0.11	0.42
Other cardiovascular disorder	-1.17	0.29
Multiple trauma	-1.11	0.45
GI bleeding	-0.42	0.44
GI surgery for neoplasm	-1.76	0.41
GI perforation/obstruction	-0.97	0.33
Other gastrointestinal disorder	-0.96	0.31

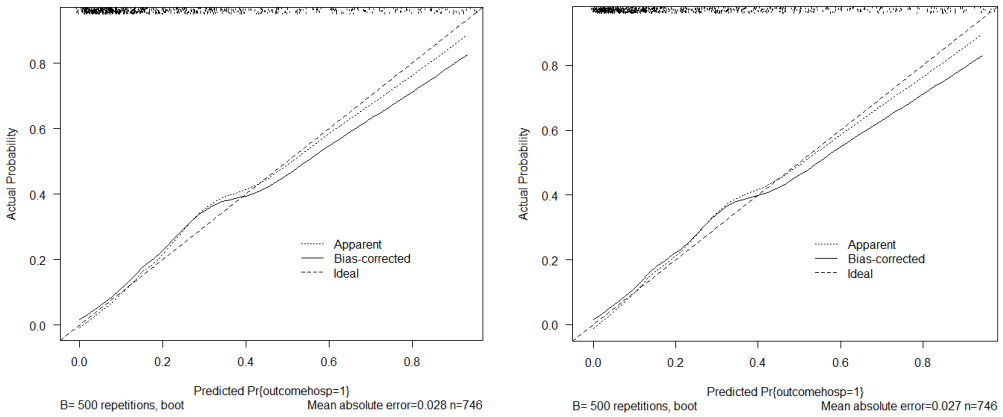

Figure E1: Calibration plot for predicted vs actual probability of hospital mortality. A: APACHE model, B: APACHE + TnI

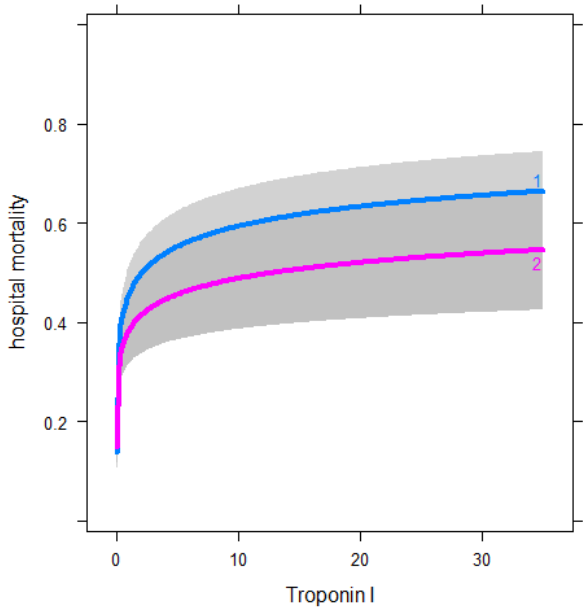

Figure E2: Association of Troponin I (mg/l) with hospital mortality with interaction term for sex. 1=Male, 2=Female. Hospital mortality appears higher in the male group, however, the 95% confidence intervals overlap, and if an interaction was present, the gradient of the two lines would differ (Interaction term for sex p=0.181).

R code:

```
# Multiple Fractional Polynomial Model ---------------------------------
library(mfp)
fit <- mfp(outcomehosp ~ fp(tn1) + fp(apscore_ad), data=tn24, family = binomial())
print(fit)
an <- anova(fit)

# logistic regression --------------------------------------------------
library(rms)
d <- tn24
dd <- datadist (d); options (datadist = "dd")

# univariable tn1
f <- lrm(outcomehosp ~ I(log2(tn1+0.001)), data = tn24, x=TRUE, y=TRUE)
plot(Predict(f, tn1, fun=plogis),
    xlab = "Troponin I",
    ylab="hospital mortality", ylim=c(-0.02, 1.02), lwd=4)

# multivariable model
f1 <- lrm(outcomehosp ~ I(log2(tn1+0.001)) + apscore_ad + emsurg + corrapiidiag,
        data = d, x=TRUE, y=TRUE)
plot(Predict(f1, tn1, fun=plogis),
    ylab="Pr[Hospital Mortality]", ylim=c(-0.02, 1.02),
    xlab = "Troponin I", lwd=4, cex.lab=14)
my.calib <- rms::calibrate(f1, method="boot", B=500) # model calibration
plot(my.calib, las=1)

##stratify by gender/interaction term

f2 <- lrm(outcomehosp ~ I(log2(tn1+0.001)) + sex + sex*I(log2(tn1+0.001)), data = d)
an <- anova(f2)
an
summary(f2,tn1=c(1,2))
plot(Predict(f2, tn1, "sex", fun=plogis),
    xlab = "Troponin I",
    ylab="hospital mortality", ylim=c(-0.02, 1.02), lwd=4)

##troponin as categorical variable
f3 <- lrm(outcomehosp ~ apscore_ad + tropcat + emsurg + corrapiidiag, data = d)

# correlation ----------------------------------------------------------

c1 <- lm(lntn1 ~ emsurg, df)
summary(c1)
sqrt(0.0095)
c2 <- lm(lntn1 ~ corrapiidiag, df)
summary(c2)
sqrt(0.1578)
c3 <- lm(lntn1 ~ apage, df)
summary(c3)
sqrt(0.02765)
c4 <- lm(df$lntn1 ~ df$aps_ad)
summary(c4)
sqrt(0.1533)
c5 <- lm(df$lntn1 ~ df$che)
summary(c5)
sqrt(0.01269)
```

```
# ROC curves -------------------------------------------------------------
library(ROCR)
#model for apiiscore, apscore_ad, tropcat, tn1 etc and ROC in derivation cohort
m1.logit <- glm (outcomehosp ~ apscore_ad + emsurg + corrapiidiag,
            family = binomial(link = "logit"), data = tn24)
m2.logit <- glm (outcomehosp ~ I(log2(tn1+0.001)) + apscore_ad + emsurg + corrapiidiag,
            family = binomial(link = "logit"), data = tn24)
m3.logit <- glm (outcomehosp ~ I(log2(tn1+0.001)),
            family = binomial(link = "logit"), data = tn24)

summary(m2.logit)
#predicted probabilities for glasgow data
predpr1 <- predict(m1.logit, data=tn24, type = "response")
m1.scores <- prediction(predpr1, tn24$outcomehosp)
predpr2 <- predict(m2.logit, data=tn24, type = "response")
m2.scores <- prediction(predpr2, tn24$outcomehosp)
predpr3 <- predict(m3.logit, data=tn24, type = "response")
m3.scores <- prediction(predpr3, tn24$outcomehosp)
predpr4 <- predict(m4.logit, data=tn24, type = "response")
m4.scores <- prediction(predpr4, tn24$outcomehosp)

# predicted probabilities for new data
predpr1 <- predict(m1.logit, newdata=tnost, type = "response")
m1.scores <- prediction(predpr1, tnost$outcomehosp)
predpr2 <- predict(m2.logit, newdata=tnost, type = "response")
m2.scores <- prediction(predpr2, tnost$outcomehosp)
predpr3 <- predict(m3.logit, newdata=tnost, type = "response")
m3.scores <- prediction(predpr3, tnost$outcomehosp)

##ROC curve with AUC
roc.perf1 <- performance(m1.scores, measure = "tpr", x.measure = "fpr")
roc.perf2 <- performance(m2.scores, measure = "tpr", x.measure = "fpr")
roc.perf3 <- performance(m3.scores, measure = "tpr", x.measure = "fpr")
plot(roc.perf1,lwd=4, col = as.list(1:10))
plot(roc.perf2, add = TRUE, lty = 2, col = "red", lwd=4)
plot(roc.perf3, add = TRUE, lty = 8, col = "green", lwd=4)
abline(a=0, b= 1, lty = 8, col = "grey")
auc1 <- performance(m1.scores,"auc")
auc2 <- performance(m2.scores,"auc")
auc3 <- performance(m3.scores, "auc")
rocobj<- roc(tnost$outcomehosp, predpr1)
ci.auc(rocobj)
rocobj1 <- roc(tnost$outcomehosp, predpr2)
ci.auc(rocobj1)
roc.test(rocobj,rocobj1)


##Multiple Imputation for routine subgroup
library(mice)
library(VIM)
imp <- mice(dfrout, method="pmm", maxit=50, seed=500, print=FALSE)
m3 <- with(imp,glm(outcomehosp ~ I(log2(tn1+0.001)) + apscore_ad + emsurg + I(as.factor(corrapiidiag)),
             family=binomial()))
m3
pooled <- pool(m3)
round(summary(pooled),2)
```

5. TROPonin I in Cardiovascular patients in CriticAL care

5.1. Introduction

Troponin release can broadly be divided into ischaemic and inflammatory causes and this introduction will explore the causes of troponin release, and the definition of Myocardial Infarction. It will then appraise some of the important perioperative and critical care studies which have used troponin. The dynamics of ischaemic (lactate) and inflammatory (C-Reactive Protein) biomarkers will be discussed, in relation to the dynamics of troponin I (TnI), in order to understand the potential mechanism of TnI release in critically ill patients with cardiovascular disease.

5.1.1. Troponin

Troponin is a 3-piece regulatory protein integral to cardiac and skeletal muscle contraction. Troponin I (TnI) and Troponin T (TnT) are isoforms specific to cardiac muscle, whereas Troponin C (TnC) is found in both cardiac and skeletal muscle. The majority of the cardiac troponin complex is bound within the cytoskeletal structure of the cardiac myocyte, however there are also free cytoplasmic components, estimated to be 3-4% for TnI and 6-8% for TnT (184). TnT is significantly bigger (37 kDa) than TnI (22kDa) (185). This cytosolic unbound pool of troponin is released first, regardless of the cause of the myocyte injury.

5.1.1.1. Potential mechanisms of Troponin release

Myocyte necrosis is the most common cause of troponin release, and includes injury from ischaemic, inflammatory, infiltrative, direct trauma and toxic causes (186). However, there is increasing evidence that troponin release is possible in the event of myocardial ischaemia, without the requirement for myocyte necrosis. This has been seen particularly in patients with supraventricular tachycardia, where there was no subsequent evidence of coronary artery disease on angiography. The formation and release of membranous blebs has also been proposed as a potential mechanism of troponin release. During ischaemia, blebs occur on the surface of the cardiac myocytes. If the ischaemia is prolonged the blebs rupture, and cellular necrosis occurs. If the ischaemia resolves before the blebs rupture, then they may be resorbed into the myocyte, or shed into the circulation and release their cytoplasmic contents without undergoing necrosis (180). Stimulation of stretch-responsive integrin in cardiac myocytes has been shown to lead to the release of intact TnI from viable cardiac myocytes in the absence of necrosis, potentially as a result of increased permeability of the cell membrane. This was associated with minimal TnI degradation, compared to TnI release from necrotic myocytes which is associated with extensive TnI degradation (187). Alternative mechanisms include apoptosis, normal myocyte cell turnover, and cellular release of proteolytic troponin degradation products (186). Regardless of the pathobiology, myocardial necrosis due to myocardial ischaemia is designated as myocardial infarction (94).

5.1.2. The diagnosis of Myocardial Infarction

According to the Third Universal Definition of Myocardial Infarction (MI), the term acute MI requires evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischaemia (80). They have defined this as follows:

Detection of a rise and/or fall of cardiac biomarker values [preferably cardiac troponin] with at least one value above the 99th percentile upper reference limit (URL) and with at least one of the following (80):

Table 9: Criteria for the diagnosis of myocardial infarction

Diagnosis of myocardial infarction
Symptoms of ischaemia New or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block (LBBB)
Development of pathological Q waves in the ECG
Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
Identification of an intracoronary thrombus by angiography or autopsy.

MI can be further categorised according to its likely cause (80):

Table 10: Categories of Myocardial Infarction

Type of Myocardial Infarction	
Type I	Spontaneous MI related to atherosclerotic plaque rupture, with intraluminal thrombus
Type II	Secondary to ischaemia, oxygen supply-demand imbalance
Type III	MI resulting in death when biomarker values are unavailable
Type IVa	MI related to PCI
Type IVb	MI related to stent thrombosis
Type V	MI related to CABG

Patients with myocardial necrosis (elevated troponin concentrations) in the absence of symptoms or signs of myocardial ischaemia are classified as having myocardial injury. Acute myocardial injury is classified where troponin concentrations display a dynamic rise and fall pattern, compared with chronic myocardial injury, where troponin concentrations remain elevated but unchanged on serial testing. Chronic myocardial injury may be found in patients with chronic heart failure, renal failure, and coronary artery disease in the community (188, 189). Patients with type II myocardial infarction and myocardial injury are a heterogeneous group with considerable overlap.

5.1.2.1. Electrocardiographic (ECG) evidence of myocardial infarction

The ECG is a key part of the diagnosis of MI, and represents regional myocardial ischaemia. It enables identification of the infarct-related artery, estimation of the amount of myocardium at risk, and determination of the therapeutic strategy (94). ECG signs associated with acute myocardial ischaemia are as follows (94):

Table 11: ECG abnormalities potentially associated with acute myocardial ischaemia. Serial ECGs must be performed to assess evolution of abnormalities.

ECG abnormality	Definition
ST elevation	New ST elevation at the J point in 2 contiguous leads: ≥0.2mV in leads V2-V3, ≥0.1mV in all other leads
ST depression/ T wave inversion	New horizontal or down-sloping ST depression ≥0.05mV in 2 contiguous leads and/or T inversion ≥0.1mV in 2 contiguous leads with prominent R wave or R/S ratio >1
Left bundle branch block	
Other ECG signs	
cardiac arrhythmias	
intraventricular and atrioventricular conduction delays	
loss of pre-cordial R wave amplitude	

Coronary artery size and distribution of arterial segments, collateral vessels, location, extent and severity of coronary stenosis, and prior myocardial necrosis can all alter the appearance of the ECG (190), and it therefore is important to compare serial ECGs. ECG signs of reperfusion represent an important marker of microvascular blood flow and subsequent prognosis. These include resolution of ST-segment elevation (191), T wave inversion within four hours of myocardial infarction, accelerated idioventricular rhythm, isolated ventricular premature depolarisations, and more rarely polymorphic ventricular tachycardia and ventricular fibrillation (190). T wave inversion that occurs during the first few hours of reperfusion therapy is a highly specific sign of reperfusion, whereas T wave inversion that develops after four hours is consistent with the evolution of the infarction.

ECG interpretation, can be challenging due to tachycardia, bundle branch blocks, non-specific changes and clinician inexperience (81). The ECG is also limited by its inadequate representation of the posterior, lateral and apical walls of the left ventricle (190).

A single ECG cannot be used to diagnose myocardial infarction as ST deviation may be observed in other conditions, such as acute pericarditis, left ventricular hypertrophy (LVH), left bundle branch block (LBBB), Brugada syndrome, stress cardiomyopathy, and early repolarization patterns (94). It is therefore essential that, particularly in patients with co-existing cardiovascular disease, serial ECGs are performed looking for dynamic ECG changes consistent with ischaemia.

5.1.2.2. Outcomes of Type II myocardial infarction and myocardial injury

Outcomes for both groups of patients are poor, and there is currently no consensus on the optimal cardiac investigation, management or treatment strategy. For consecutive unselected hospital inpatients outwith the critical care setting, patients with type II MI or myocardial injury have worse outcomes than patients who present with type I MI, with a third of patients dead at one year (192), and 60% of patients with Type II and 75% of patients with myocardial injury dead at five years (193). However, this reflects all-cause mortality, and it is not known whether therapeutic intervention can improve outcomes. Patients with Type II MI were twice as likely as those with myocardial injury to be admitted with a type I MI in one year, suggesting that a proportion of patients with Type II MI may benefit from further investigation and treatment for coronary artery disease.

5.1.2.3. Diagnostic thresholds in patients with suspected Acute Coronary Syndrome

Lowering of the diagnostic threshold from 200ng/l to 50ng/l with the use of sensitive TnI assays in 2011 inevitably led to an increase in the diagnosis of myocardial infarction (194). However, it also improved clinical outcomes: recurrent myocardial infarction and death were reduced from 39% to 21% at one year. This was thought to be due to improved clinical management of patients with suspected ACS and small TnI rises. These patients had been less likely to be referred to cardiology, prescribed dual antiplatelet therapy, considered for revascularization or commenced on secondary preventative therapies despite presenting with chest pain and having evidence of myocardial ischaemia on ECG. However, patients classified into Type II MI or myocardial injury underwent more investigations and utilized additional cardiac services without altering their poor clinical outcome.

The use of highly sensitive troponin assays, with limits of detection x10-100 lower than previous generation contemporary assays, is now increasingly common in clinical practice. The latest guidelines recommend the use of assays with optimal precision at the 99th centile, defined as a coefficient of variation of less than 10%. This has enabled revision of the diagnostic thresholds of TnI for myocardial infarction based on the upper reference limit (99th centile) of healthy individuals, and is now sex-specific, reflecting the larger left ventricular mass in men compared with women (men 0·034mg/l, women 0·016mg/l) (170). Increased sensitivity may reduce specificity for the diagnosis of myocardial infarction, with the potential for misdiagnosis and inappropriate cardiac interventions or medication. These assays have been able to detect troponin in studies of patients in the community with stable cardiovascular disease, including stable angina (188) and congestive cardiac failure (189). In an analysis of the Scottish Heart Health Extended Cohort (SHHEC), 74.6% of the population had TnI>1.9ng/l (195). The assays have also enabled detection of cTnT and cTnI in non Acute Coronary Syndrome states such as sepsis, pulmonary embolus, tachycardia, anaemia, and critical illness (83).

5.1.3. Non-cardiac mechanism of TnI elevation

5.1.3.1. Sepsis

Sepsis and inflammation are the most common non-cardiac causes of troponin elevation in critically ill patients (196, 197). Troponin elevation is associated with higher catecholamine requirements, lower stroke work index and left ventricular ejection fraction, and a higher mortality (198). Approximately 50% of patients with sepsis develop ventricular dysfunction, and levels of cTn correlate with the severity of left ventricular (LV) systolic dysfunction (83, 199). Ver Elst et al found an elevated TnI in 50%, and TnT in 36% of patients with sepsis (n=46). There was no increase in myocardial necrosis at post-mortem in patients with previously elevated troponin levels. They were unable to conclude whether myocardial damage was a cause or consequence of LV dysfunction (200). Landesberg et al found that troponin elevation correlated most closely with left ventricular diastolic dysfunction and right ventricular dilatation (201).

Significant associations have been noted between left ventricular dysfunction in sepsis, troponin elevation and release of the inflammatory cytokines tumour necrosis factor alpha (TNF-alpha) and interleukin-6 (IL-6) (202). This suggests that cytokines may cause direct myocardial injury, potentially by a nitric oxide mediated mechanism, resulting in left ventricular depression and troponin release (183, 203, 204). However, Landesberg

found no correlation between inflammatory cytokines and systolic or diastolic myocardial dysfunction in sepsis (205). Injection of endotoxin into healthy volunteers has not increased systemic troponin levels (206).

Ostermann et al found that TnT levels were independently associated with markers of systemic inflammation (IL-6) and ventricular dilatation (NT-pro-BNP) (183), suggesting that troponin release during critical illness may represent both systemic inflammation and ventricular strain.

The coagulation cascade is activated in sepsis, and disseminated intravascular coagulation is relatively common. It is possible that this hypercoagulable state may result in coronary plaque rupture and thrombosis (Type I MI). However, Altmann et al found no difference in coagulation parameters between troponin positive and negative patients with sepsis, and significant flow-limiting coronary artery disease was ruled out in 64% of patients (202). Activation of coagulation may also play a key role in the pathogenesis of microcirculatory alterations (207). Fibrin deposition occurred in a significant proportion of capillaries in mice that were challenged with endotoxin, and the addition of antithrombin decreased the number of non-perfused capillaries (208). However, microthrombi formation is rarely documented in experimental sepsis (209).

Oxygen supply/demand imbalance is common in critical illness, and may result in myocardial necrosis (Type II MI), particularly in the presence of underlying critical coronary artery disease. Sepsis demands an increase in cardiac output, which increases the demand of the myocardium for oxygen. At the same time, oxygen delivery is reduced, either by respiratory failure, anaemia, systemic hypotension or microcirculatory dysfunction. Hypotension have been associated with myocardial injury and troponin elevation in sepsis (210, 211). However, thermodilution catheters placed in the coronary sinus in patients with sepsis have shown preserved coronary artery blood flow, net myocardial lactate extraction, and no increase in the difference between coronary artery and sinus oxygenation (83). This suggests there are multifactorial causes.

5.1.3.2. Pulmonary embolism

Troponin elevation in pulmonary embolism is well recognised, particularly for saddle embolism (212, 213). The peak cTnT level occurs around eight hours, is lower than for acute MI, and falls more quickly (214). This suggests a different mechanism: it may be as a result of right ventricular strain (215), or right ventricular impairment and high pulmonary resistance reducing left ventricular filling (216). Raised troponin levels are associated with increased mortality in both haemodynamically stable and unstable patients (217). Troponin is already used clinically as a prognostic marker in pulmonary embolism (158).

5.1.3.3. Chronic Obstructive Pulmonary Disease (COPD)

Soyseth et al quote a four fold increase in troponin T elevation 4.26 (95%CI 3.02-6.00) in acute exacerbations of COPD (218). In the stable COPD population, increasing Troponin T was associated with an increased leucocyte count and serum creatinine, and falling FEV_1. Troponin elevation may be due to pulmonary hypoxic vasoconstriction, with subsequent right heart strain similar to pulmonary embolism. Other potential mechanisms include co-existing coronary artery disease, hypoxaemia with subsequent type 2 MI, and systemic inflammation (219).

5.1.3.4. End stage renal disease (ESRD)

TnI and TnT are frequently raised in patients with stable end-stage renal disease (ESRD) and no evidence of acute coronary syndrome (220). In fact, in Jacobs et al's cohort of patients with ESRD, he found cTnT levels

over the 99th reference centile in 100% of patients (221). This may be as a result of co-existing CAD or left ventricular hypertrophy, stretch mediated troponin release, or ongoing subclinical myocardial injury due to uraemia and impaired excretion (222). TnT is elevated significantly more than TnI in ESRD, which may be as a result of the kidneys failing to clear its larger molecular weight (223). Studies in ICU comparing TnI and TnT have shown that TnI is a better prognostic marker in renal failure. TnI is less stable in the blood, and is more prone to chemical changes in addition to higher rates of clearance in dialysis (224).

5.1.3.5. Brain Injury

TnI elevation has been reported in 20-68% of patients after subarachnoid haemorrhage (SAH) (225). This is thought to be secondary to the catecholamine storm induced by traumatic brain injury (TBI) and SAH, and driven by the central neuroendocrine axis, which massively increases sympathetic outflow and activates the adrenal glands. Damage to the insular and the hypothalamus also results in activation and subsequent dysfunction of the autonomic nervous system with an intense inflammatory response that results in the release of cytokines, adhesion molecules, and other multifunctional peptides (226). Excess local release of noradrenaline from myocardial sympathetic nerve terminals can also result in ECG changes, arrhythmias, left ventricular dysfunction and troponin release, "neurogenic stunned myocardium" (226). ECG changes are common after SAH, particularly ST segment changes, flat or inverted T waves, prominent U waves, and prolonged QTc (227). Contraction band necrosis has been found in up to 50% of patients with fatal SAH at post mortem, and results from excessive exposure to catecholamines and cellular calcium entry, leading to a hypercontracted state (228). Most patients with left ventricular dysfunction after SAH have had normal coronary arteries at angiography or autopsy (228).

5.1.4. Critical Care myocardial injury studies

A systematic review by Lim et al in 2006 looked at the prevalence of raised troponin in patients in general ICUs using conventional troponin assays. They found the prevalence of raised troponin over 20 studies and 3278 patients was 12-85%, median 43% (IQR 21-59%). Elevated troponin was associated with longer hospital stay, and higher all-cause mortality (37.1% vs13.6% [OR, 3.88; 95% CI, 3.28-4.60; P<0.001]). The OR for mortality in patients with sepsis and an elevated troponin was 3.49 (95%CI 1.73-7.04) (159).

Lim also performed two prospective cohort studies and found that myocardial infarction (raised troponin levels and contemporaneous ischaemic ECG changes) occurred in 26-36% of ICU patients. Hospital mortality in patients with MI was 43%, compared to 27% in those with elevated troponin only. More than half of the MIs diagnosed by prospective screening were missed by the clinical team, although the associated mortality was similar irrespective of whether the events were recognised or not (39% vs. 35% ICU mortality and 50% vs. 35% hospital mortality, respectively, with non-significant p-values) (229).

This study was repeated by Ostermann et al, who used a highly sensitive troponin T assay in 144 non-cardiac patients in ICU (95). They also investigated the association between raised troponin T and sepsis. The researchers found 84% (121) of patients had at least one raised cTnT (>15ng/l). Of these patients, 20 (14%) had ECG changes consistent with a definite MI, 39 (27%) had ECG changes consistent with a possible MI, and 62 (43%) had elevated troponin levels only. Only 20% of definite MIs were recognised by the clinical team. On the

day of troponin elevation, 69% of patients with troponin elevation had sepsis. Their ICU mortality was 29%, compared with 9% for patients who were not septic.

5.1.5. Perioperative myocardial injury studies

Nagele investigated the prognostic capability of hs-cTnT on mortality in 608 high cardiovascular risk patients after major non-cardiac surgery (97). The study found that before surgery, nearly all patients (98.5%) had a detectable level of hs-cTnT (>5ng/l), and that 41% were over the 99th reference centile (>14ng/l). Factors associated with raised hs-cTnT were diabetes, congestive heart failure, coronary artery disease, hypertension and chronic renal failure. 82% of patients had significant hs-cTnT rises post-operatively. Pre-operative hs-cTnT was a strong predictor of post-operative rise of hs-cTnT, acute MI, and also for 3 year mortality (25% 3 year mortality amongst patients with pre-operative hs-cTnT>14ng/l).

The VISION Group have conducted one large prospective cohort, presented in two parts, in patients aged 45yrs or older, looking at Myocardial Injury after Non-cardiac Surgery (MINS) (160, 179). Their first study used non high-sensitivity TnT and comprised 15,065 patients (179). They found that Troponin I >0.03ng/ml was an independent predictor of 30d mortality HR 3.87 (95%CI 2.96-5.08), and they therefore defined MINS as Troponin I >0.03ng/ml judged due to myocardial ischaemia (96). ECG evidence of ischaemia was not necessary for diagnosis. They found that 1200 patients (8.0%) suffered MINS, and that 58.2% of these patients would not have fulfilled the universal definition for myocardial infarction. They adjusted for patient age, cardiovascular comorbidity, and type of surgery, but not severity of illness. They suggest that patients who suffer MINS may benefit from aspirin and statin therapy.

The second VISION cohort of 21,842 patients aimed to determine the association between perioperative high sensitivity TnT and 30 day mortality, and potential diagnostic criteria for MINS based on hsTnT (160). They found that peak post-operative TnT was significantly associated with 30-day mortality, compared with reference <5ng/l: 20-65ng/l mortality rate 3%, HR 23.63 (95% CI 10.32 to 54.09); 65-1000ng/l: mortality rate 9.1%, HR 70.34 (95% CI 30.60 to 161.71); and >1000ng/l: mortality rate 29.6%, HR 227.01 (95% CI 87.35 to 589.92). They also concluded that elevated postoperative TnT without an ischaemic feature was also associated with 30-day mortality. Compared to No Injury, the HR for Injury was 3.20 (95% CI 2.37 to 4.43), and for Infarction the HR was 5.04 (3.56 to 7.12). They used this to define MINS as an elevated postoperative hsTnT level judged as resulting from myocardial ischaemia without the requirement of an ischaemic feature.

Both VISION cohorts used an elevated TnT as evidence for Myocardial Injury after Non-cardiac Surgery, with the justification that these patients have higher 30 day mortality. However, they have used TnT to improve perioperative risk estimation, rather than being able to use TnT to determine whether patients have had a myocardial infarction. In the second cohort (160), expert unblinded physicians assessed patient notes for presence of ischaemic features, and for evidence that the TnI was due to a post-operative non-ischaemic aetiology (classified as sepsis, pulmonary embolus, atrial fibrillation, cardioversion, chronic elevation). They adjudicated that only 481 (11.0%) patients had peak TnT>20 due to non-ischaemic postoperative complications. However, although they have included emergency vs elective surgery in their logistic regression analysis, they have not looked further into these potentially different mechanisms. Patients requiring emergency (<24hours after acute surgical condition) or urgent (24-72hrs) may well have had a systemic inflammatory response

syndrome (SIRS), which may be associated with TnT elevation, due to direct cytokine injury or "TnT leak" (183). They found no significant additional risk of 30 day mortality with ischaemic features on ECG (HR 5.04, 95% CI 3.56 to 7.12) compared with TnI elevation alone (HR 3.20, 95% CI 2.37 to 4.32), however the rate of TnT elevation with ischaemic changes in this population was only 271 (6.9%), and the 30 day mortality was only 316 (1.4%) it may be that the study was underpowered to detect the effect of ischaemia. Furthermore, 30 day mortality may be too early to see mortality from myocardial infarction, and longer term mortality may be more useful. Other outcomes such as length of stay in hospital and patient related outcome measures may be more relevant, such as health related quality of life. It follows that the authors' recommendations to consider all these patients for aspirin and statin therapy, may be misplaced and too broad. Aspirin and statins may reduce risk in patients with intra-coronary plaques, but they are unlikely to improve risk in patients where the mechanism of TnT release is non-ischaemic. This may in fact put patients at higher risk, due to the side-effects of aspirin and statins.

A cohort study of the POISE (PeriOperative Ischemic Evaluation) trial (n=8,351) also looked at the characteristics and short-term prognosis of perioperative myocardial infarction in patients undergoing noncardiac surgery (230). They defined perioperative MI as elevated cardiac biomarkers and one or more of: ischaemic symptoms, ischaemic changes on ECG, coronary artery intervention (percutaneous coronary intervention, coronary artery bypass graft surgery), evidence of MI on cardiac imaging, or evidence of acute MI at autopsy. 5.0% had perioperative MI within 30 days, and a further 8.3% had isolated elevation of cardiac biomarkers. 30 day mortality was 11.6% among patients who had a perioperative MI and 2.2% among those who did not. The odds of mortality at 30 days were 4.76 (95%CI 2.68 to 8.43) for patients with ischaemic symptoms, and 4.00 (95% CI 2.65 to 6.06) for those without. The highest quartile (a troponin or creatine kinase-MB level ≥3.6 times the upper limit of normal) for isolated cardiac biomarker elevation was also an independent predictor of 30 day mortality (adj OR 2.54 95%CI 1.65 to 3.90). The investigators suggest that secondary prophylaxis cardiac interventions (such as a statin or angiotensin-converting enzyme inhibitor) are reasonable in the absence of perioperative MI RCTs. They found that the use of aspirin was associated with a 46% reduction in the 30 day mortality rate in those suffering a perioperative MI, and statins were associated with a 76% reduction.

Van Waes et al also conducted a prospective perioperative MI study (n=2232) in non-cardiac surgery (231). The prevalence of myocardial injury was 19%, and TnI was an independent predictor of 30 day mortality: minor (70-590ng/l) TnI increase RR 2.4 (95% CI 1.3 to 4.2), and 10- to 100-fold increase in TnI was RR 4.2 (95% CI 2.1-8.6). They have also attributed TnI rise to ischaemic causes - coronary plaque rupture, thrombosis of coronary arteries or myocardial oxygen supply-demand imbalance - and have not considered alternative inflammatory mechanisms. Only 34.9% of patients were referred to cardiology, and only 13.6% of patients with TnI elevation had any cardiological intervention (medication 13.6%, angiography 2.2%).

Both VISION and van Waes report that troponin is an independent predictor of mortality at 30 days, and present its release as an ischaemic event. The recommendations suggested by these studies, that patients should be started on cardiac medication do not take into account the different mechanisms of troponin release. Furthermore, if troponin does represent severe yet stable disease in the presence of an exacerbating condition

that may result in later cardiac complications, cardiologists are often reluctant to intervene in patients with stable coronary artery disease during the unstable phase of the postoperative period.

5.1.6. Diagnosis of MI in critical illness

Diagnosis of MI in patients with critical illness is not straightforward. Many patients are unable to communicate any symptoms due to sedation and ventilation, strong analgesia, distracting injuries, and delirium. TnI elevation, as described, is common in critical illness, and in addition to cardiac causes, may also potentially be raised due to cytokines in sepsis, renal failure, or acute neurological disease (80, 83). ECGs are not performed routinely, and ECG interpretation is difficult due to tachycardia, arrhythmias and non-specific changes. Two groups have looked at ECGs taken routinely in heterogeneous critically ill patients (81, 232, 233). They found that ECG interpretation had poor agreement for the presence of myocardial ischaemia or infarction. This was improved to moderate agreement once the ECG was interpreted alongside the patient's troponin values. Specific ECG changes such as bundle branch block had high reliability, compared to non-specific T wave flattening.

Bedside imaging is limited to transthoracic echocardiography which may miss small but important regional wall motion abnormalities – an injury involving >20% of myocardial wall thickness may be required to detect a wall motion abnormality (234).

5.1.6.1. Diagnosis of MI in critically ill patients with co-existing CVD

Patients with co-existing CVD are at high risk of further myocardial injury. Surgery and trauma induce an inflammatory state, with increase in the concentrations of cytokines such as TNF-alpha, Interleukin-1, Interleukin-6, and CRP. Patients are hypercoagulable due to increases in PAI-1, factor VIII, and platelet reactivity, and decreases in antithrombin III concentrations. Furthermore, patients have increased catecholamine and cortisol levels as a result of the stress they are undergoing. All of these may lead to coronary artery shear stress, plaque fissuring, and subsequent acute coronary thrombosis, or Type I MI (235). Even in the absence of plaque rupture, increased oxygen demand and reduced oxygen delivery in the presence of stable atherosclerotic stenosis may result in Type II myocardial infarction.

It is important to attempt to delineate the mechanism of raised TnI in critically ill patients with CVD, in order to identify patients where cardiac or coronary investigations or therapies may be indicated. For the patient who presents with sub-massive pulmonary embolism, TnI elevation may be secondary to right ventricular strain or hypoxia, and coronary angiography is both unwarranted and an unnecessary risk. For the patient who presents with community acquired pneumonia, chest pain and ECG changes, TnI elevation may be due to hypoxia, tachycardia or hypotension, with the acute illness representing a physiological stress test. In this context, it is appropriate to diagnose acute MI.

5.1.6.2. Interventions to prevent MI in critically ill patients with co-existing CVD

There have been no studies in critical care aimed at minimising the oxygen supply-demand imbalance with the rate of myocardial infarction as an end-point. These include interventions aimed at increasing oxygen delivery such as the use of higher blood transfusion thresholds, or higher mean arterial pressure; and decreasing myocardial oxygen demand such as the prevention of tachycardia with beta blockade. These are key areas for future research.

5.1.6.3. Management of MI in critically ill patients with co-existing CVD

If Type I MI is suspected, then invasive coronary angiography should be considered. However if TnI elevation is in the context of oxygen supply-demand imbalance, then the need for further investigation and treatment is uncertain. A survey of 310 intensivists regarding treatment strategies for critically ill patients with elevated troponin and without typical symptoms of MI or ECG changes found that 76% would start aspirin or clopidogrel, 47.4% would start heparin, 48.9% would start high dose statins, 68.7% would start beta-blockers and 37.6% would use an ACE-inhibitor. 72.7% would request a cardiology consultation, and 51.3% would refer for an angiogram once the patient was stable (236). Furthermore, patients with CVD are frequently already on secondary prevention therapies such as anti-platelets and statins, and monitoring and follow-up in recovery may be the most appropriate management. It is plausible that manipulation of haemodynamic parameters to minimise the myocardial oxygen supply-demand imbalance, with interventions described above may reduce further damage to the myocardium.

5.1.6.4. Biomarker release in critically ill patients with co-existing CVD

Unlike other hospital specialties, there is substantial heterogeneity of presenting diagnoses in critical care, from elective surgery, to major trauma, to pneumonia. Furthermore, the timing of admission to ICU may vary dependent on timing of patient presentation to hospital services, local hospital practice and bed occupancy rates as well as severity of illness: ICU admission cannot be used accurately to define "time of onset of critical illness". Unlike in the patient presenting with chest pain, when biomarkers are taken six and twelve hours after the onset of pain, patients are at different stages in their critical illness when they are admitted to ICU, and biomarkers will also be at different stages of release. The dynamics of troponin will be discussed in relation to ischaemic (lactate) and inflammatory (C-Reactive Protein) biomarkers.

5.1.6.5. Biomarker dynamics

5.1.6.5.1. Troponin

Following acute myocardial infarction, troponin is released in three forms: a ternary complex (Tn T-I-C), a binary complex (Tn I-C) and free troponin T (184). There is significant heterogeneity in the cross-reactivity of antibodies to the various forms of TnI, which is reflected in the differences in absolute TnI measurements between assays (184). Time to peak TnI after ST elevation myocardial infarction is 11.8h (10.7–11.8) followed by a nearly log-linear decrease (237). In these patients, TnI elevation correlates with infarct size and mortality. The initial rise is due to the release of cytosolic TnI (238). The true half-life of TnI is less than 2h, however TnI remains elevated after acute MI for five to seven days, most likely due to the ongoing release of TnI from the myofibril-bound fraction (239). The half-life of troponin clearance is significantly shorter in patients with non-Q wave infarcts compare to those with Q wave infarcts (6.8 +/- 5.6h vs 20.4 +/- 10.7h) (238). However, kinetics of TnI in patients with critical illness (and potentially different aetiologies of TnI elevation) have not been studied.

5.1.6.5.2. C-Reactive Protein (CRP)

CRP is an acute phase protein that is raised in inflammation and sepsis (240). C-reactive protein binds to several polysaccharides and peptido-polysaccharides present in bacteria, fungi and parasites in the presence of calcium. These complexes activate the classical complement pathway, acting as opsonins and promoting phagocytosis (241). CRP is synthesised by the liver, mainly in response to interleukin 6 (IL-6) which is produced in both

infection and inflammation (242). Synthesis is also mediated by tumour necrosis factor α (TNF α) and IL-1 β (243).

The median concentration of CRP is 0.8mg/l (IQR 0.3-1.7mg/l) in the healthy human population, and is below 10mg/l in 99% of normal samples (244). The secretion of CRP begins four to six hours after the stimulus. It doubles every eight hours, and peaks at 36-50 hours (240). CRP has a half-life of 19 hours, and therefore falls rapidly with removal of the stimulus: persistent elevation implies persistence of the stimulus. CRP concentration is not affected by renal failure, or renal replacement therapy (245). CRP is typically significantly elevated in bacterial infections (both gram-positive and gram-negative), and frequently lower in viral infections. Other conditions commonly seen in ICU that cause CRP elevation include trauma, burns, surgery, tissue necrosis and blood transfusion.

5.1.6.5.3. Lactate

Glycolysis in the cytoplasm produces the intermediate metabolite pyruvate, and under aerobic conditions, pyruvate is converted to acetyl CoA to enter the Kreb's cycle. Under anaerobic conditions, pyruvate is converted to lactate, which can then be utilised locally or released into the bloodstream. The normal concentration of lactate is 0.5-1mmol/l. Lactate has a half-life of around 20 minutes, and is cleared by the liver. Raised lactate levels in critically ill patients are traditionally associated with anaerobic glycolysis secondary to systemic oxygen supply-demand imbalance (246). At a critical level of oxygen delivery, oxygen consumption becomes limited by oxygen delivery, and this coincides with a sharp increase in lactate levels (247).

Lactate levels correlate with the severity of the overall oxygen debt and survival, and are used to monitor perfusion as resuscitation proceeds (248). Lactate has been shown to be more closely related to outcome than standard haemodynamics, including oxygen delivery and consumption (247). Lactate has also recently been added to the ICNARC case mix programme, which looks at predictors of hospital mortality in the first 24 hours of ICU admission (167). Analysis of the Surviving Sepsis Campaign database found that patients with lactate values greater than 4mmol/l and hypotension had significantly increased mortality (44.5%) compared to a reference group of lactate less than 2mmol/l and no hypotension (mortality 29.0%) (249). Trzeciak et al found that hospital mortality rates for emergency patients with infection with “high” lactate (≥4.0 mmol/l) were 38% compared with 15% for “low” lactate (0.0-2.0 mmol/l), and 25% for “intermediate” (2.1-3.9 mmol/l) (250). They found that an initial lactate ≥4.0 mmol/l was associated with sixfold higher odds of acute phase death.

Recent trials have directed therapy towards the normalisation of lactate (251-253), or included normalisation of lactate as part of a bundle of early goal directed therapy (254, 255). These have shown a moderate reduction in mortality with lactate-guided resuscitation compared to normal care, or $ScvO_2$ normalisation strategy.

However, in critically illness, elevated lactate may not always be a result of global arterial hypoxia, and there are several reasons why lactate could increase under aerobic conditions. High inflammatory states such as sepsis are associated with direct inhibition of pyruvate dehydrogenase by endotoxin and accelerated aerobic glycolysis in skeletal muscle secondary to epinephrine-stimulated Na+, K+-ATPase activity (256, 257). Hepatic failure and renal failure may decrease the clearance of lactate (256, 258). Sepsis also impairs mitochondrial function, related to nitric oxide and peroxynitrites (258). Increased lactate production in aerobic conditions is also seen in lung injury, alkalosis, and some drugs and toxins (such as metformin, methanol, and cyanide) (258).

Resuscitation that targets persistently high lactate levels may result in harm from excess inotropes or blood transfusion.

5.2. Study Aims and Objectives

5.2.1. Primary Aim

To determine prospectively the dynamic changes in Troponin I levels in critically ill patients with pre-existing cardiovascular disease, across a range of critical illnesses.

5.2.2. Secondary Aim

To determine whether it is possible to distinguish between myocardial infarction and other causes of TnI elevation in critically ill patients with cardiovascular disease.

5.2.3. Objectives

1. To determine the incidence of myocardial infarction and myocardial injury as defined by the Third Universal Definition of myocardial infarction.
2. To explore the duration of TnI elevation above baseline with respect to mechanism of injury (cardiomyocyte necrosis vs reversible ischaemia).
3. To explore the relationship between TnI and biomarkers representing global inflammation (C-Reactive Protein, CRP) and global ischaemia (lactate).
4. To understand the incidence of significant anaemia and its management in critically ill patients with cardiovascular disease, and its relationship with TnI
5. To determine the independent variables associated with TnI elevation.
6. To explore whether myocardial injury has an independent association with the outcomes of critically ill patients with CVD.
7. To explore the relationship between myocardial injury, mortality and haemoglobin.

5.3. Methods

5.3.1. Study summary

Patients were consented and then enrolled in the study at admission to ICU. Over the subsequent ten day period we collected baseline demographic data, and daily information regarding illness severity/organ support, myocardial infarction, and blood transfusion. We retrieved blood samples from biochemistry that had already been taken for routine purposes, and ran the Abbott highly sensitive Troponin I assay. We recorded the results of blood tests that had been routinely taken. No additional blood tests were taken for the purposes of this study.

We performed a daily ECG for the duration of the patient's stay in ICU for a maximum of five days. This was performed at the patient's bedside in ICU by a trained clinician.

After discharge from ICU, we recorded date of hospital discharge, mortality status at six months, and date of death if it was appropriate.

5.3.2. Study design

TROPICCAL was a prospective cohort study.

5.3.3. Study setting

The study was undertaken in Scotland in the ICUs of the Royal Infirmary Edinburgh, the Western General Hospital, Edinburgh, and the Queen Elizabeth University Hospital, Glasgow. In England the study took place in the Critical Care Units of: Newcastle Hospitals NHS Foundation Trust (The Freeman Hospital and the Royal Victoria Infirmary), East Lancashire Hospital Trust, University Hospital of South Manchester, John Radcliffe Hospital Oxford, Salford Royal NHS Foundation Trust, Guys and St Thomas' Hospital London, Bolton NHS Foundation Trust, Portsmouth Hospitals NHS Trust and Brighton and Sussex University Trust. These are multi-disciplinary adult closed level 3 ICUs.

5.3.4. Participants

We recruited patients between 11th February 2015 and June 30th 2016. Inclusion criteria were consecutive critically ill adults with pre-existing CVD (as defined in Table 12) requiring admission to ICU.

Table 12: Definition of Cardiovascular Disease for TROPICCAL

Cardiovascular Disease	The collective term for all diseases affecting the heart and blood vessels
Acute Coronary Syndrome	Acute Myocardial Infarction (MI) Anginal symptoms Electrocardiographic changes consistent with ischaemia Biomarker elevation
Chronic Cardiac Disease	Ischaemic Heart Disease Left/Right/Congestive Cardiac Failure Valvular disease (non-endocarditis associated) Chronic arrhythmia on treatment Hypertensive heart disease Hypercholesterolaemic/hyperlipidaemic heart disease
Cerebrovascular Disease	Cerebrovascular Accident (CVA) Transient Ischaemic Attack (TIA)
Peripheral Vascular Disease	Abdominal Aortic Aneurysm (AAA) +/- Thoraco- (TAAA) Vascular surgery Symptoms of vascular insufficiency under vascular review
Age >75 with Diabetes or Hypertension	Type I or II Diabetes on medication Hypertension on medication

Patients were excluded for the following reasons: traumatic brain injury, acute stroke or intracranial haemorrhage as these are associated with TnI rise and ECG changes, but without oxygen supply-demand imbalance. We similarly excluded patients who were post cardiac arrest, post cardiac surgery, or traumatic myocardial injury as they could have TnI release from direct trauma. Lastly we excluded patients who were receiving palliative care, transferred from another ICU, age under 18 years, or expected stay in ICU less than 48 hours. Patients with new ACS were excluded if this was the primary presenting diagnosis, but were eligible if their main reason for admission was non-cardiac (for example: a patient presenting with pneumonia requiring intubation and ventilation, who also had symptoms or signs of ACS would be eligible for inclusion).

5.3.4.1. Participant Selection and Enrolment

In the ICUs in which this project was undertaken, members of the research team were embedded within ICU, and were closely integrated with the clinical team. In many cases they were also members of the clinical team.

All admissions to Intensive Care were screened for eligibility into the study by members of the critical care research team or the routine healthcare team. A screening log was maintained at each site to capture reasons for non-inclusion of eligible patients. The research team approached the potential participant or relative/next of kin/welfare attorney directly to discuss involvement in the study. If interested in participating, the appropriate information sheet was provided and a maximum of 24 hours given to consider the study and ask any questions. If interested, consent was sought for inclusion in the study. Many of these patients were, by the nature of their illness, deemed incapacitated to provide consent and in this instance the laws for adults with incapacity differed in Scotland and England. In Scotland, the patient's guardian/welfare attorney/nearest relative was approached for consent either in person or via telephone. If the approach was by telephone, the initial call came from a member of the clinical team who asked if the patient's guardian/welfare attorney/nearest relative would be willing to speak to the research team. In England, if the nearest relative (personal consultee) was present on the ICU, they were approached for agreement. If the relative was not present on the ICU, an independent consultant (nominated consultee) was approached for agreement. In cases where there was no personal consultee, or he/she

was not contactable, the patient remained in the study based on the nominated consultee agreement, but we made every effort to contact the personal consultee.

Patients who were deemed capable of consent by the clinician in charge were given a patient information leaflet to read which fully explained the study with its risks and benefits. This was discussed with them and consent sought by the chief investigator or appropriately trained member of the team.

For most patients admitted to hospital, the case notes had clear information relating to the identity of the guardian/welfare attorney/nearest relative. Contact details for a first contact (and additional contacts) were usually documented in the unitary patient record (a key document in the patient's medical notes), and/or in the Emergency Department sheet. The patient's guardian/welfare attorney/nearest relative was identified from this information. No incapacitated patient was entered into the study without consent (agreement in England) from a guardian, welfare attorney or relative (or independent consultant in England). We approached the guardian/welfare attorney/nearest relative during a visit to the ICU. Direct consent of these individuals was preferable, however witnessed phone consent were acceptable in Scotland, with the individual signing the consent form at the earliest opportunity. In such cases where telephone consent were obtained, a verbal description of the study was provided, with an opportunity to ask any questions. This process was witnessed by a member of the clinical team who was not connected with the study.

Once the patient recovered capacity, they were provided with a Patient Information Sheet and asked to provide retrospective consent. The patient was given sufficient time to consider the information given. If the patient refused consent, the patient was asked to specify whether they would allow data collected so far to be entered into the analysis and ECGs and blood samples to be retained. In the situation that the patient did not consent to any data collected to be used, no data was entered into the analysis and no further data was collected. In the situation that the patient did not consent to the retention of the ECGs and blood samples, these were destroyed.

Participants had the right to withdraw from the study at any point. Participant information collected during the study was treated in the strictest confidence. The study did not interfere with daily care or treatment of any of the patients.

5.3.5. Data Collection

All data was entered directly onto a secure, trial specific database using REDCap: Research Electronic Data Capture (Vanderbildt University, USA). This was a secure, password protected platform, hosted on University of Edinburgh servers. All identifiable data was removed before the dataset was extracted for analysis. The following data was collected from all participants recruited onto the study:

Baseline: date of admission, demographic information, admission type, admission diagnosis, severity of illness score (APACHE II), cardiovascular disease or risk factors, cardiac medications, comorbidity on admission to ICU (APACHE II comorbidity, Functional Comorbidity Index, see 5.3.7.6.3 Comorbidity) (259), duration of stay in hospital prior to ICU admission. More than one cardiovascular inclusion criterion could be entered per patient (eg chronic cardiac disease AND peripheral vascular disease).

Day 0-10: An ECG was performed within six hours of ICU admission (either before or after admission), and then daily for a maximum of five days. ECGs were not performed once the patient was discharged from ICU.

Each ECG was reviewed by 2 cardiologists for signs of ischaemia or infarction. In the event of disagreement, a third cardiologist reviewed the ECG. The cardiologists were blinded to the patient's clinical details including TnI data. ICU clinicians looking after the patients were given a copy of the study's ECGs.

Daily measures of severity of illness were recorded whilst the patient remained in ICU. These included mechanical ventilation, inotropic support, and renal support.

Other relevant blood tests (haemoglobin, platelet count, renal function, prothrombin time, lactate) were obtained from paper/electronic medical records.

Clinically relevant information was recorded including presence/absence of blood transfusion, and clinically diagnoses myocardial infarction.

5.3.6. Follow up

Patients were prospectively followed up for ten days by the research team. Hospital records were checked for hospital length of stay and mortality, and six month mortality by the research team.

5.3.7. Variables

5.3.7.1. Troponin I

We used the ARCHITECT STAT high-sensitive troponin I assay (Abbott Diagnostics, Illinois, USA), which is a two-step immunoassay using chemiluminescent microparticle (CMIA) technology and reaction is measured in relative light units (RLUs). A direct relationship exists between the concentration of TnI in the sample and the RLUs detected by the ARCHITECT *i* System optics. The concentration of TnI is read relative to a standard curve established with calibrators of known TnI concentrations. The capture antibodies are directed at epitope residues 87-91 and 24-40, and the detection antibodies are directed at epitope residue 41-49. It offers increased precision for measuring very low plasma troponin concentrations and can quantify troponin concentrations in 98% of healthy persons with a limit of detection of 1.2 ng/l and 10% co-efficient of variation at concentrations <5.5 ng/l (260) considerably lower than the ARCHITECTSTAT troponin I assay (limit of detection 10ng/l, CV 10% at 50ng/l) (170).

Daily TnI blood tests were retrieved from existing blood samples. Samples were processed locally in real time at the following hospitals which had the ARCHITECT STAT high-sensitive troponin I assay: Royal Infirmary Edinburgh, the Western General Hospital, Edinburgh, the Queen Elizabeth University Hospital, Glasgow, University Hospital of South Manchester, John Radcliffe Hospital Oxford, and Bolton NHS Foundation Trust. All TnI assays were performed within 72 hours of being drawn from the patient. We collected samples from sites that did not have the Abbott Highly Sensitive Troponin Assay, and stored them at -80C for batch analysis of TnI in NHS Lothian. Hospitals that stored TnI were Newcastle Hospitals NHS Foundation Trust (The Freeman Hospital and the Royal Victoria Infirmary), East Lancashire Hospital Trust, Salford Royal NHS Foundation Trust, Guys and St Thomas' Hospital London, Portsmouth Hospitals NHS Trust and Brighton and Sussex University Trust. In these hospitals, specimens were collected from routine blood samples which were stored at 2-8°C within 72 hours.

We have presented TnI as a percentage of the peak TnI, and centred the time axis around the day of peak TnI, due to the wide range of peak TnI values, and the variation in the timing of peak TnI.

We explored the univariable relationship between admission TnI and peak TnI using linear regression, with a log transformation for both admission and peak TnI.

5.3.7.2. Objective 1: To determine the incidence of myocardial infarction and myocardial injury as defined by the Third Universal Definition of myocardial infarction.

5.3.7.2.1. ECG interpretation

ECG interpretation was independently performed by three senior cardiology research fellows, who each had at least ten years of postgraduate medical experience. All were blinded to patient name and clinical information. Each patient's ECGs were reviewed by a cardiologist. To assess for agreement, the first 56 patients' ECGs were reviewed by two cardiologists. For the admission ECG, the cardiologists were asked to classify the ECG as normal/abnormal, and to classify abnormalities as ST elevation or presumed new left bundle branch block (LBBB), ST depression, T wave inversion, pathological Q waves and RBBB. These parameters were chosen a priori, and are a standard approach to reading ECGs for acute changes potentially associated with ischaemia (94). These abnormalities may be chronically present on ECGs in patients with co-existing cardiovascular disease, and their presence on one ECG does not therefore indicate acute ischaemia. Dynamic changes on serial ECGs are essential for the diagnosis of myocardial ischaemia. They were also asked to comment on the rhythm: normal sinus rhythm, sinus rhythm with heart rate >100 beats per minute, atrial fibrillation or flutter, other supraventricular tachycardia, or $2^{nd}/3^{rd}$ degree heart block. For subsequent ECGs, the cardiologists were asked if it differed from the admission ECG, and abnormalities were classified as above. They were also asked to give an overall impression of whether the changes on each patient's serial ECGs represented ischaemia.

ECG agreement was assessed using Cohen's kappa statistic (k<0.20 poor agreement; 0.21 – 0.4 fair agreement; 0.41 – 0.60 moderate agreement; 0.61 – 0.80 good agreement, 0.81 – 1.00 very good agreement (261)).

5.3.7.2.2. Classification of Myocardial Injury Category

We restricted the dataset to the first 5 days after ICU admission, as ECG analysis was performed for these days. We would have been unable, therefore, to differentiate infarction from injury using days 6-10.

In line with the Third Universal Definition, we classified TnI elevation as follows:

Myocardial Infarction:

- a rise and fall of ≥20% in cTnI over the sex-specific diagnostic threshold (16ng/l for women, 34ng/l for men)

AND

- dynamic changes on ECG, classified as ischaemic by cardiologists blinded to clinical details.

Myocardial Injury (Acute): a rise and fall of ≥20% TnI over the sex-specific diagnostic threshold, with no dynamic changes on ECG.

Myocardial Injury (Chronic): a persistently elevated TnI over the sex-specific diagnostic threshold, with a rise/fall <20%, and no dynamic changes on ECG.

No Injury: no TnI elevation, no ECG changes.

Other: no TnI elevation, ECG changes.

We collapsed "No Injury" and "Other" into a single "No Injury" category. "Other" consisted of patients with dynamic ECG changes consistent with ischaemia, but a peak TnI below the sex-specific diagnostic threshold. The majority of these patients had T wave inversion, which had poor agreement between cardiologists. These patients had similar baseline characteristics and outcomes to the "No Injury" group, and were therefore grouped together.

We performed two sensitivity analyses.

Higher TnI threshold:

There is little literature regarding higher thresholds in the presence of already raised cardiac biomarkers. Previous consensus reports suggested a threshold of x3 for creatine kinase (CK) after percutaneous intervention, and x5 for CK after cardiac surgery (262, 263). The third universal definition for MI requires a TnI x5 the URL of normal after percutaneous intervention, and x10 after cardiac surgery (94). We have restricted thresholds to five times the upper reference level and questioned whether a more significant rise in TnI might be more specific for Infarction. We looked at the fit and the discriminative ability of the new myocardial injury categories in the multivariable logistic regression model for mortality at six months. This enabled us to comment on the potential consequences of raising the threshold.

Criteria for ECG ischaemia:

For this sensitivity analysis we excluded T wave inversion from our definition for ischaemia on ECG. This was because there was poor agreement amongst our cardiologists for T wave inversion, and it was associated least with elevated TnI (see 5.4.4 ECG agreement). Furthermore, the literature suggests that early T wave inversion may represent reperfusion and not ischaemia (190).

5.3.7.3. Objective 2: To explore the duration of TnI elevation above baseline with respect to mechanism of injury (cardiomyocyte necrosis vs reversible ischaemia).

Duration of TnI elevation: TnI displays a log-linear decrease after ST elevation MI (237), with a return to baseline within 72 hours. A shorter duration may potentially represent reversible myocardial ischaemia (release of cytosolic TnI), and a longer duration may represent necrosis and/or a persistent insult. We hypothesised that patients with a short duration of TnI elevation might be more likely to be in the Injury category, and patients with a longer duration of elevation might be in the Infarction category. We also explored whether a longer duration of elevation was associated with worse mortality at six months. We explored the duration of TnI elevation using a fall to ≤25% of the peak value as a surrogate baseline, or a fall to lower than the sex-specific diagnostic threshold, whichever occurred first. Patients who had fewer than five days of TnI results after the peak TnI, and whose TnI did not fall below the threshold or ≤25% of peak value were not included in this analysis as we were unable to adequately comment on the descent. Patients who had five days or more of TnI results after the peak TnI where there was evidence of TnI descent but not to below the threshold or ≤25% of peak value were censored as "11 days" (i.e. the maximum possible value in our dataset). As an extreme case analysis, we stratified by patients whose TnI dropped to threshold or ≤25% of peak value on day 1 ("Early")

with those who dropped on or after day 4 ("Late"). We explored the duration of TnI elevation across the categories of myocardial injury using the Kruskal Wallis Test. We looked at the association between duration of TnI rise and mortality at six months using the chi^2 test.

5.3.7.4. Objective 3: To explore the relationship between TnI and biomarkers representing global inflammation (C-Reactive Protein, CRP) and global ischaemia (lactate).

5.3.7.4.1. Biomarker representing Inflammation: C-Reactive Protein (CRP)

We measured CRP in all patients in hospitals that sent their assays to St John's Hospital, Livingston for analysis: Newcastle Hospitals NHS Foundation Trust (The Freeman Hospital and the Royal Victoria Infirmary), East Lancashire Hospital Trust, Salford Royal NHS Foundation Trust, Guys and St Thomas' Hospital London, Portsmouth Hospitals NHS Trust and Brighton and Sussex University Trust

Daily samples were obtained when clinical samples were taken from the patient for up to ten days during and after ICU admission. Specimens were collected from routine blood samples which were stored at 2-8C within 72 hours. Samples were centrifuged at a relative centrifugal force of 3000-3500xg for 30 minutes, and >0.5ml serum was removed and stored in aliquot tubes in a freezer at -80C. Samples were transferred to NHS Lothian on dry ice, and stored at -80C. Samples were thawed, placed in a new container, and centrifuged. 0.3ml was then removed for analysis, and the remaining sample was re-frozen. The same samples were analysed for both CRP and troponin.

The time to peak CRP is 36-50 hours after insult. TnI and CRP were taken from the same sample. To look for a potential causal relationship between CRP ("inflammation") and TnI, we restricted the dataset to the time of peak TnI + 48hrs following peak TnI. We performed univariable linear regression with peak TnI as the log-transformed dependent variable. We examined CRP across the Myocardial Injury categories, using the Kruskal-Wallis test.

5.3.7.4.2. Biomarker representing ischaemia: Lactate

Lactate was recorded in all sites, from samples taken for clinical indication. We recorded the worst (highest) lactate level in the 24 hours preceding the troponin sample, as measured on an arterial blood gas.

In line with previous studies, and current guidelines, we categorised a severe elevation in lactate as ≥4.0 mmol/l "High" (250, 254, 264), "Middle" 2.0-3.9 mmol/l and "Normal" as <2.0 mmol/l.

Lactate appears in the bloodstream contemporaneously with end-organ hypoxia. The time to peak TnI is 11.8 hours after ST elevation, although this has not been studied in patients with critical illness. To look for a potential causal relationship between ischaemia and TnI we centred the dataset around the time of peak TnI. We divided time into 3 categories: Group "1" 48 hours preceding peak TnI (when there is biological plausibility that ischaemia reflected by lactate could cause a peak in TnI); Group "0" greater than 48 hours preceding peak TnI, and Group "2" after peak TnI (where lactate and TnI are less likely to be causally related). We log10-transformed peak lactate, and performed repeated measures ANOVA between groups 1 and 2, and 0 and 1 to see if there was a significant difference in the peak lactate between the biologically plausible Group 1, and the other groups.

We further explored Group 1, looking at the distribution of peak lactate in the 48 hours preceding peak TnI, across the Myocardial Injury categories (no injury, injury, infarction). We used the Kruskal-Wallis test to test for statistical significance.

5.3.7.5. Objective 4: To understand the incidence of significant anaemia and its management in critically ill patients with cardiovascular disease, and its relationship with TnI

We categorised severe anaemia as Hb<90g/l. We divided our groups into "High" (patients whose Hb never fell below 90g/l), "Middle" (nadir Hb 70-90g/l) and "Low" (nadir Hb<70g/l). This reflects the uncertainty for likely transfusion triggers in patient with CVD (<70g/l: definitely transfuse, >90g/l: unlikely to transfuse). Transfusion of one unit of red blood cells at a time is recommended for stable anaemic patients in ICU. To look at the transfusion trigger in non-bleeding patients, we excluded patients who received more than two units at a time, and used the lowest Hb recorded on that day.

We looked at the distribution of haemoglobin in the 24 hours preceding peak TnI, and across the Myocardial Injury categories (No injury, Injury, Infarction). We used the Kruskal-Wallis test to test for statistical significance.

5.3.7.6. Objective 5: To determine the independent variables associated with TnI elevation.

We pre-selected a range of variables considered potentially important as risk factors for TnI elevation.

5.3.7.6.1. Age

Age was entered as a continuous variable in all models. We divided age by 10 for estimates of relative risk or odds as this is clinically more meaningful.

5.3.7.6.2. Diagnosis

Diagnoses were recorded as APACHE III diagnostic codes in Scotland, and ICNARC diagnostic codes in England. We mapped APACHE III codes onto ICNARC codes. Due to the low frequency within each sub-category, and the requirement to reduce the categories for entry into regression models, we classified diagnosis by ICNARC system: "respiratory", "cardiovascular", "gastrointestinal", "genito-urinary" and "other". Acute intracerebral pathology was an exclusion criterion, but we still included patients admitted after back, or neck surgery or injury who were classified by ICNARC or APACHE as "neurological".

For the analysis adding TnI to the APACHE II model (as per Chapter 3), we mapped APACHE III and ICNARC diagnoses onto APACHE II diagnoses.

5.3.7.6.3. Comorbidity

We recorded severe comorbidity using APACHE II comorbidities (liver: biopsy proven cirrhosis, documented portal hypertension; cardiovascular: New York Heart Association Class IV; respiratory: chronic restrictive, obstructive or vascular disease resulting in severe exercise restriction or documented chronic hypoxia, hypercapnia, secondary polycythemia, severe pulmonary hypertension, or respirator dependency; renal: receiving chronic dialysis; immunocompromised: immunosuppression, chemotherapy, radiation, steroids, leukaemia, lymphoma, AIDS).

We also recorded less severe comorbidity using the Functional Comorbidity Index. This has 18 binary categories (Arthritis, Osteoporosis, Asthma, Chronic obstructive pulmonary disease (COPD) or acquired

respiratory distress syndrome (ARDS) or emphysema, Angina, Congestive heart failure, Heart attack, Neurological disease, Stroke or TIA, Peripheral vascular disease, Diabetes, Upper gastrointestinal disease, Depression or Anxiety or panic disorder, visual impairment, Hearing Impairment, Degenerative disc disease, Obesity), and was developed by Groll et al and used physical function as the outcome rather than mortality (259). It correlates with the physical component score from the SF-36 health related quality of life questionnaire, and has been used and validated in ICU populations previously (265).

5.3.7.6.4. Cardiac medication

We recorded the presence/absence of the following cardiac medication: beta blocker, angiotensin converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker, calcium channel blocker, diuretic, anti-platelet, warfarin, statin, other. We recorded whether the patient was on the medication long-term, leading up to their ICU admission. We did not record whether the medication was continued during their ICU stay, nor whether it was restarted after discharge from ICU.

5.3.7.6.5. Severity of illness scoring

The use of risk prediction models in ICU is standard, and enables benchmarking of practice and outcomes across units (164). We have used two methods to score severity of illness:

5.3.7.6.5.1. APACHE II score

The APACHE II score (Acute Physiological and Chronic Health Evaluation II) is a measure of patient severity of illness within the first 24 hours of admission to ICU (165). It combines 12 routine physiological variables with chronic health status and age to provide a general measure of disease severity (Chapter 3). This data is routinely entered by clinical staff in ICU in Scotland, and instructions were given to the research staff in England on how to calculate it. The range is 0-71, and an increasing score is associated with subsequent risk of hospital death. The APACHE II score can then be combined with presenting diagnosis and requirement for emergency surgery to give a predicted mortality (Table 13). We kept APACHE II in as a continuous variable. In addition, we categorised patients into "Less Severely Ill" and "More Severely Ill", using the median APACHE II Score as the cut value.

Table 13: APACHE II Score with approximate hospital mortality interpretation. Adapted from http://reference.medscape.com/calculator/apache-ii-scoring-system

APACHE II Score	**Predicted Hospital Mortality (%)**	
	Non-operative	Post-operative
0-4	4	1
5-9	8	3
10-14	15	7
15-19	24	12
20-24	40	30
25-29	55	35
30-34	~73	~73
>35	85	88

5.3.7.6.5.2. SOFA Score

The SOFA Score (Sequential [or "Sepsis"] Organ Failure Assessment) score is not restricted to the first 24 hours of ICU admission, and can be performed on a daily basis (266). It is made up of six variables, each representing

an organ system. The worst physiological parameter for each variable in a 24 hour period is assigned a score 0-4, with a maximum total score of 24 (Table 14).

Table 14: SOFA Score with approximate hospital mortality interpretation. Adapted from http://clincalc.com/icumortality/sofa.aspx

Maximum SOFA Score	Predicted Hospital Mortality (%)
0-6	<10
7-9	15-20
10-12	40-50
13-14	50-60
15	>80
>15	>90

5.3.7.7. Readmission to ICU

Patients could only be entered into the study once, during their index ICU admission. If the patient was readmitted to ICU within 48 hours of ICU discharge, this was counted in the first ICU stay. Other ICU readmissions were included in the hospital length of stay.

5.3.7.8. Outcomes

The main outcomes were peak Troponin I (ng/l), and 6 month mortality.

5.3.8. Bias

5.3.8.1. Selection bias

We excluded patients who were admitted with primary cardiac diagnoses (acute ST elevation Myocardial Infarction, cardiogenic shock, pericarditis, myocarditis) and non-cardiac diagnoses which are known to be associated with TnI elevation. These included diagnoses of myocardial trauma (blunt, penetrating, including cardiopulmonary resuscitation), acute intracerebral pathology (acute intracerebral bleed, infarction, or trauma), and of primary myocardial infarction. We also excluded patients in whom treatment was being withdrawn, as we felt it was inappropriate to approach them or their relatives. Finally, we did not include patients who were expected to stay less than 48 hours in ICU, as we would have minimal information regarding severity of illness scoring, or ECGs. We are therefore unable to generalise our findings to these patients.

5.3.9. Statistics and Data Analysis

5.3.9.1. Study size

We initially planned to recruit 150-200 patients, limited by the likely recruitment rate of critically ill patients with cardiovascular disease in NHS Lothian. However, TROPICCAL was adopted onto the CRN portfolio in England, and we were therefore able to extend our study to other sites and continue recruitment for 18 months. In this analysis, we have excluded patients recruited after 30th June 2016 who were recruited specifically for our echo substudy.

5.3.9.2. Missing data

We had minimal missing data. Our three main variables of interest for our multivariable analyses were TnI, APACHE score and lactate. TnI was missing in six (2.2%) patients due to miscommunication with local laboratories, APACHE score was also missing in six (2.2%) patients, due to lack of local data entry, and lactate in the 24 hours before peak TnI was missing in 10 (3.6%) patients. We recorded lactate from routinely taken

arterial or venous blood samples, and these patients did not have lactate taken in the 24 hours before peak TnI. We did not think that this influenced our outcomes, and we have concluded that the data were missing at random (MAR). We used multivariable imputation by chained equation to impute multiple possible values for each missing value (267). This is superior to single imputation in that it takes into account the uncertainty in missing value imputation. We imputed five datasets. We used predictive mean matching for continuous variables, and logistic regression for binary variables. Variables included in the imputation were predictive of missingness, associated with the variable being imputed and the outcome variable of the analysis (268).

5.3.10. Statistical analysis

All data were analysed using R (R Core Team v3.3.2, Vienna, Austria) (172). Statistical significance was set at p=0.05. All tests were 2-sided. We presented continuous data as mean (95% CI), or median (IQR) if non-normally distributed. Binary data was presented as frequency (%). For univariable comparisons, we used the following statistical tests: Parametric variables were compared using the Student t test or ANOVA, and non-parametric variables were compared using the Mann Whitney U or Kruskal-Wallis test. Categorical data were compared using Fisher's exact test.

The time to peak TnI post MI is approximately 11.8 hours. Examination of our dataset confirmed the time from baseline to peak was less than 24 hours. In the prediction of peak TnI we therefore restricted the dataset to the 24 hours preceding peak TnI for each patient. The distribution of peak TnI was positively skewed, and we therefore log transformed this, using base 2 for ease of clinical interpretation. We performed ordinary least squares regression using the R rms package (175). We performed backwards stepwise regression, retaining variables if they were statistically significant ($p<0.05$) or were clinically significant despite not reaching statistical significance. We added an interaction term for SOFA and haemoglobin as we hypothesised that the impact of reduced oxygen delivery (anaemia) might be greater in patients who were more severely ill, compared with those who were less severely ill. We have presented pooled coefficients from our multiple imputation datasets.

5.3.10.1. Objective 6: To explore whether myocardial injury has an independent association with the outcomes of critically ill patients with CVD.

5.3.10.1.1. Univariable analyses:

5.3.10.1.1.1. Kaplan Meier plots

We used Kaplan-Meier plots to assess survival up to six months stratified by baseline characteristics in univariable analyses. Continuous variables were grouped in order to assess the survival curves. Haemoglobin was grouped by potential transfusion thresholds (<70g/l, 70-90g/l, >90g/l). Lactate was grouped by "Normal" <2.0mmol/l, "Middle" 2.0-4.0mmol/l, "High" >4.0mmol/l. APACHE II Score and TnI were grouped by quartiles. Univariable associations were reported using the log rank test for non-ordinal categorical variables. We looked at whether there was an interaction between TnI and ischaemia. We categorised TnI into a binary variable, with the cut point at the median. We then stratified by the presence or absence of ischaemia, and performed the log-rank test as well as visual inspection of the KM plots for these subgroups.

5.3.10.1.2. Multivariable analysis

Our primary aim was to assess whether myocardial injury was an independent predictor of mortality after adjustment for confounders.

We used logistic regression as we had complete follow up for all of our patients. We entered myocardial injury as a three level categorical variable ("No Injury", "Injury" and "Infarction"). The interaction between TnI and ischaemia is built into this categorisation. We also added an interaction term for myocardial injury category and severity of illness as we believed that the mechanism of TnI release may be different dependent on severity of illness. We used APACHE score at ICU admission as our measure of illness severity – this already includes age, comorbidity, and type of admission, and we have not therefore included these variables separately. This reduced the number of degrees of freedom, an important consideration given the size of the dataset.

We have reported the odds ratios for APACHE Score per point difference, lactate per mmol/l, and Hb per 10g/l.

5.3.10.1.3. Sensitivity analysis: TnI and ECG ischaemia

Our secondary aim was to assess whether there was a difference between patients who were diagnosed with Injury, compared with Infarction, given the limitations of ECG interpretation. Given the shape of early exploratory plots, and assessment of linearity of TnI, we were concerned that patients with Infarction had higher mortality than patients with Injury not because of added ischaemia, but because they typically had higher peak TnI. We kept TnI as a continuous variable and added an interaction term with ischaemia. We entered peak TnI as a continuous variable, and we used multiple fractional polynomials to find the transformation of best fit. We checked this with visual inspection of log odds plots. We stratified our analyses by the presence/absence of ischaemia, and then entered an interaction term for ischaemia and TnI in the same model. The odds ratio for TnI is presented as the difference between 1st Quartile (25ng/l) and 3rd Quartile (375ng/l).

5.3.10.1.4. Sensitivity analysis: Exclusion of T wave inversion from Infarction diagnosis

Our blinded cardiologists reported only fair agreement for dynamic T wave inversion on ECG, and we found that T wave inversion was the least predictive dynamic ECG change of high peak TnI. We therefore undertook a sensitivity analysis where we excluded T wave inversion from the criteria for Infarction. This means that we were unable to use the binary ischaemia variable. Instead, we used the dynamic abnormalities reported by the cardiologists, and classified as non-ischaemic patients where T wave inversion was the only abnormality.

We re-ran the OLS model for peak TnI with the new definition of ischaemia, looking at the association of peak TnI with ischaemia. To look at mortality up to six months, we produced Kaplan Meier plots stratified by the new myocardial injury variable and performed the log rank test for univariable analysis. We have re-run the logistic regression model with the new variable, looking at overall fit and discriminatory ability of the model, as well as the odds ratios for myocardial injury.

5.3.11. Ethical Practice

The following Research Ethics Committees reviewed this study and gave a Favourable Opinion: Scotland A Research Ethics Committee (Scotland) and Newcastle and North Tyneside Ethics Committee (England).

5.4. Results

Between February 15th 2015 and 30th June 2016 we recruited 282 patients to TROPICCAL (Figure 8, Figure 9). We screened 7,617 admissions to ICU in 11 different NHS trusts. 1,787 (23.4%) had CVD and were potentially eligible for TROPICCAL (Table 15). Of these patients, 1,454 (81.4%) met at least one exclusion criteria, and 49 (2.7%) patients declined consent (Table 16). Three patients withdrew consent after enrolment. Baseline demographic data were available for all 279 patients. Troponin samples were not processed for six patients due to miscommunication with local laboratories.

Prevalence of CVD varied considerably between units, from 13.1% (Salford Royal Infirmary), to 34.9% (Portsmouth) (Table 15).

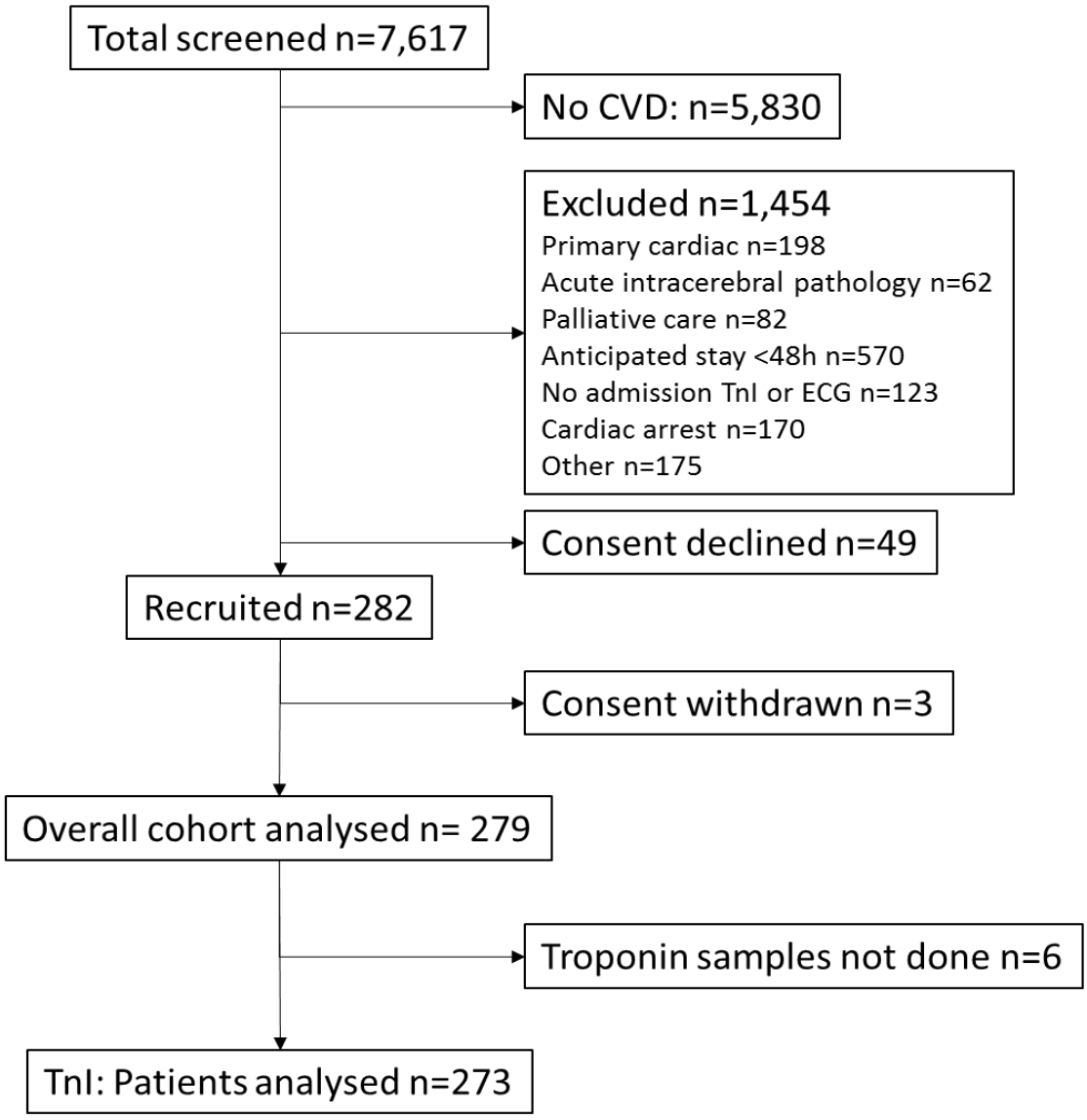

Figure 8: Recruitment to study

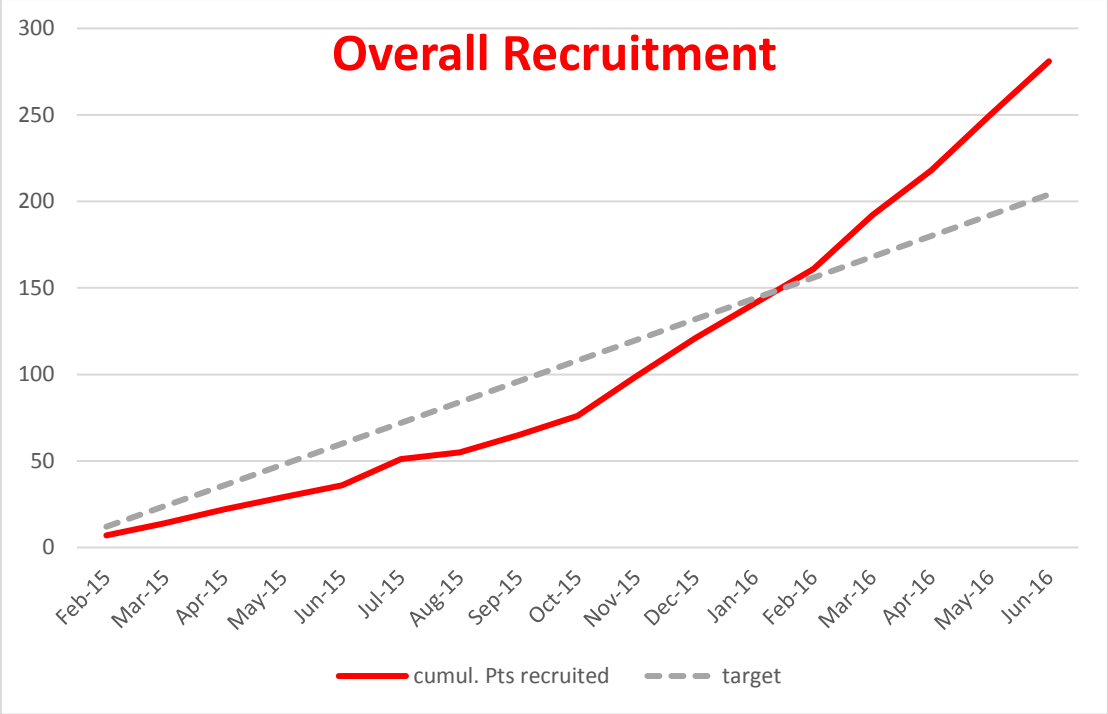

Figure 9: Monthly overall recruitment

Table 15: Recruitment to TROPICCAL by NHS Trust site up to June 30th 2016

	Total screened	Pts with CVD	%	Recruited
Bolton	208	45	21.6	6
Brighton	173	26	15.0	8
GSTT	1295	225	17.4	46
ELHT	1091	334	30.6	23
Newcastle	1119	304	27.2	18
Salford	507	70	13.8	11
UHSM	368	113	30.7	22
Lothian	1769	296	16.7	117
Oxford	582	167	28.7	18
Portsmouth	496	173	34.9	11
Glasgow	108	34	31.5	2
Total	7716	1787	23.2	282

Table 16: Reasons for Exclusions at screening per site. Other: no staff available to screen within appropriate time frame, unable to contact relatives, too drowsy to consent, severe dementia, age<18, recruited to another study, from out of region.

	Primary cardiac	**Acute intracerebral pathology**	**Palliative RIP**	**Anticipated stay <48hr**	**No admission TnI, ECG**	**Cardiac arrest**	**Consent declined**	**Other**
Bolton	2	3	4	12	16	8	2	2
Brighton	1			11		6		
GSTT	106	2	7	15		13	9	27
ELHT	16	10	25	154	67	25	9	4
Newcastle	14	33	12	159		20	7	17
Salford	6	4	5	9	22	2		11
UHSM	3	1	6	33	18	13	2	14
Lothian	31	1	10	34		42	15	48
Oxford	9	6	8	46		17	4	47
Portsmouth	7	2	8	86		13	1	2
Glasgow	3		4	11		11		3
Total	198	62	89	570	123	170	49	175

5.4.1. Baseline Characteristics

The mean age of patients was 72.3 years, and 71.7% were male. The most common inclusion criterion was "Chronic cardiac disease" (62.4%), although age ≥75 with diabetes or hypertension was also common (32.5%). 79.9% of admissions were emergency admissions (emergency surgical 29.0%, emergency non-operative 50.9%). The most frequent diagnoses at presentation were gastrointestinal (30.1%), respiratory (23.3%) and cardiovascular (22.9%). Most patients (83.5%) had no APACHE comorbidities, however, the prevalence of less severe comorbidity as measured by the FCI (functional comorbidity index) was much higher, and half the patients had 2 or more comorbidities (median 2, IQR 1,3).

At admission to ICU, the median APACHE II score was 18 (IQR 15, 23), and the SOFA score was 7 (5, 10), corresponding to a predicted mortality of 15-20%. 37.3% of patients were on vasopressor support, and 48.2% of patients were ventilated within the first 24 hours of admission, very few patients received renal replacement therapy. The median Hb was 111g/l (93, 125), and the median TnI was 25.7g/l.

Table 17: Baseline Characteristics for whole cohort. n=279. Patients can appear in more than one CVD category.

Variable	n or mean/median	% or SD or IQR
	279	
Age mean(SD)	72.3	10.9
Male sex n(%)	200	71.7
CVD n(%)		
ACS	22	7.9
Chronic cardiac disease	174	62.4
CVA/TIA	42	15.1
PVD	55	19.7
>75, DM/BP	90	32.5
System diagnosis n(%)		
Respiratory	65	23.3
CVS	64	22.9
GI	84	30.1
Neuro	12	4.3
Genito-urinary	31	11.1
other	22	7.9
Comorbidity		
Apache: 0	233	83.5
1	36	12.9
≥2	10	3.6
FCI med(IQR)	2	1,3
Type of admission n(%)		
Elect Surgical	56	20.1
Emerg Surgical	81	29
Non-operative	142	50.9
Severity of Illness in first 24h		
APII score	18	15,23
SOFA score	7	5,10
Vasopressors n(%)	104	37.3
During ICU stay	196	70.3
Mech Vent n(%)	134	48.2
During ICU stay	185	66.3
RRT n(%)	8	2.9
During ICU stay	61	21.9
pre ICU LOS (d) med(IQR)	1	0,2
Hb at admission g/l med (IQR)	111	93,125
nadir Hb	84	75,100
Troponin at admission ng/l med (min, IQR, max)	26	1, 7 ,56, 2157
Peak Troponin ng/l (min, IQR, max)	105	3, 25, 375, 58820
ECG ischaemia n(%)	78	28

5.4.2. Cardiac medication

There were 24 (8.6%) patients who were on no cardiac medication (Table 18). Overall, 41.2% of patients were on a beta-blocker, 39.4% were on an Angiotensin Converting Enzyme Inhibitor (ACE-I), 55.9% of patients were on an anti-platelet or warfarin, and 49.5% of patients were on a statin. 21 (31.3%) patients with chronic coronary artery disease were not on anti-platelet or warfarin therapy prior to admission.

Table 18: Chronic cardiac medication by cardiovascular subgroup. Patients could present with ACS in the context of a different main diagnosis.

	n	No meds		Beta blocker		ACE-I		Anti-plt/warfarin		Statin	
		%		%		%		%		%	
Total	279	24	(8.6)	115	(41.2)	110	(39.4)	156	(55.9)	138	(49.5)
Inclusion Criteria											
ACS	22	0	(0.0)	10	(45.5)	8	(36.4)	14	(63.6)	14	(63.6)
CCD	174	11	(6.3)	87	(50.0)	70	(40.2)	115	(66.1)	82	(47.1)
CVA/TIA	42	5	(11.9)	19	(45.2)	12	(28.6)	30	(71.4)	21	(50.0)
PVD	55	6	(10.9)	18	(32.7)	21	(38.2)	32	(58.2)	31	(56.4)
75 DM/BP	90	4	(4.4)	31	(34.4)	38	(42.2)	44	(48.9)	45	(50.0)
Chronic Cardiac Disease (CCD) Subcategories											
CAD	67	3	(4.5)	32	(47.8)	28	(41.8)	46	(68.7)	44	(65.7)
CCF	27	2	(7.4)	13	(48.1)	10	(37.0)	21	(77.8)	13	(48.1)
Valvular	26	4	(15.4)	12	(46.2)	8	(30.8)	20	(76.9)	9	(34.6)
Arrhythmia	65	2	(3.1)	39	(60.0)	28	(43.1)	49	(75.4)	32	(49.2)
Hypertensive	58	2	(3.4)	30	(51.7)	25	(43.1)	42	(72.4)	22	(37.9)
Hyperchol	13	1	(7.7)	7	(53.8)	4	(30.8)	9	(69.2)	10	(76.9)

5.4.3. Troponin I

Table 19: Baseline Characteristics for TnI at admission (n=252), and for peak TnI (ng/l) (n=273)

	Admission TnI Median	min,Q1,Q3,max	Peak TnI Median	min,Q1,Q3,max
n	252		273	
TnI (ng/l)	36	(1, 13, 140, 45400)	114	(3, 27, 412, 58820)
Inclusion Criteria				
ACS	41	(2, 20, 238, 45440)	367	(7, 49, 1432, 47080)
Chronic Cardiac	31	(1, 13, 112, 45440)	82	(3, 23, 305, 58820)
CVA/TIA	36	(2, 13, 126, 45440)	90	(4, 26, 363, 47080)
PVD	23	(1, 9, 122, 2885)	101	(3, 24, 615, 18380)
>75 with DM/BP	72	(2, 20, 194, 19860)	135	(3, 44, 491, 58820)
Admission type (ng/l)				
El Surgery	19	(1 ,6, 43, 588)	37	(3, 19, 206, 58820)
Em Surgery	38	(1, 15, 161, 15810)	139	(3, 35, 651, 39600)
Medical	48	(1, 14, 190, 45440)	112	(3, 31, 370, 47089)
Sex (ng/l)				
Male	31	(1, 10, 121, 45440)	94	(3, 25, 352, 58820)
Female	51	(1,15, 152, 6968)	120	(4, 39, 496, 14840)
Severity of Illness				
APACHE II<=18	19	(1, 8, 54, 45440)	43	(3, 16, 166, 58820)
APACHE II >18	82	(1, 23, 233, 19860)	169	(3, 69, 685, 19860)
No organ support	41	(1, 13, 149, 19864)	81	(3, 20, 227, 47085)
Mech Ventilation	41	(1, 13, 157, 6968)	148	(3, 41, 662, 18382)
Vasopressor	39	(1, 13, 150, 45440)	149	(3, 44, 921,58822)
RRT	29	(8, 23, 54, 204)	278	(18, 109, 899, 18382)
ICU mortality				
Survivors	31	(1, 11, 125, 45440)	88	(3, 25, 355, 58820)
Non-survivors	69	(2, 24, 273, 6968)	169	(11, 69, 554, 9292)

There were 273 patients who had TnI samples available for analysis. 252 (90.3%) patients had TnI taken on the day of ICU admission. All patients had detectable TnI. There was a wide range of TnI at admission (median 36ng/l, Q^0, Q^1, Q^3, Q^4: 1, 13, 140, 45400) (Table 19), and peak TnI (median 114, Q^0, Q^1, Q^3, Q^4: 3, 27, 412, 58820) (Table 19,

Figure 10). 64 (84.2%) women and 130 (66.0%) men had peak TnI over the sex-specific diagnostic threshold for diagnosis of myocardial infarction (overall total n=194 (71.1%)

Figure 10).

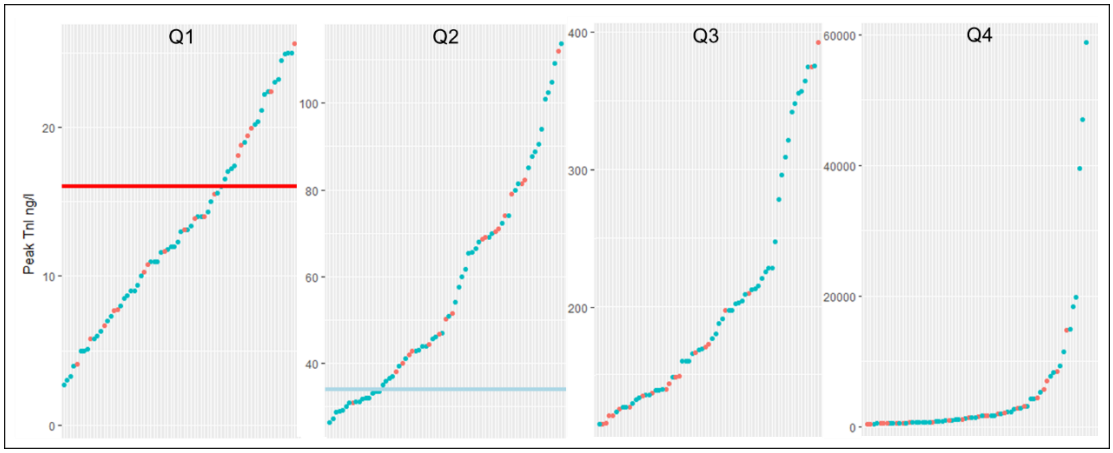

Figure 10: Peak Troponin I (ng/l) for each patient in ascending magnitude of TnI. Wide range of TnI, therefore displayed in Quartiles. Q1 TnI 3-26ng/l; Q2 26-114; Q3 114-393; Q4 393-58822. Blue=Male (n=197), Red=Female (n=76). Horizontal lines at sex specific thresholds (Male=Blue 34ng/l, Female=Red 16ng/l,). Males>34ng/l n=130 (66.0%), females>16ng/l n=64 (84.2%). Total patients with Peak TnI over sex-specific diagnostic threshold n=194 (71.1%).

Patients who presented with clinically recognised ACS in addition to their main presenting diagnosis had higher peak TnI than patients who presented without ACS (Table 19, Figure 11). Patients could appear in more than one chronic cardiac disease subgroup. Male and female patients had similar median admission and peak TnI, although the maximum admission and peak TnI were much higher for male patients. Patients who were admitted after elective surgery or with low APACHE II scores had considerably lower admission and maximum TnI than patients who were admitted as emergencies and those patients with APACHE II scores >18. Patients who were receiving organ support had similar TnI at admission to patients without organ support, but the peak TnI was considerably higher. ICU survivors had lower admission and peak TnI than ICU non-survivors.

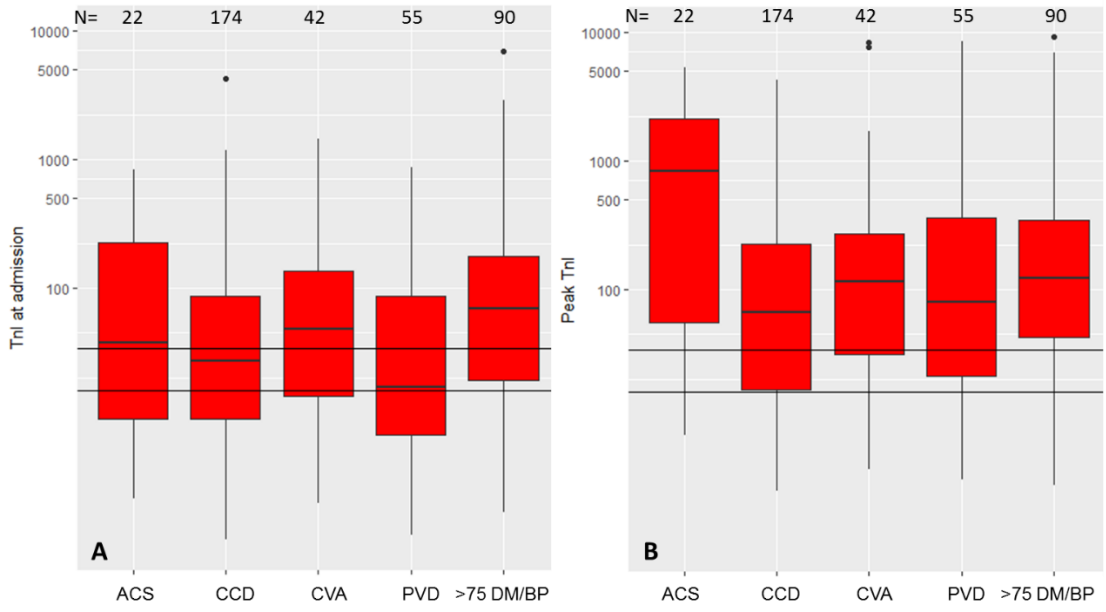

Figure 11: CVD subgroup by TnI (log 10 scale). A: TnI at admission, B: peak TnI. Horizontal lines at sex specific thresholds (16ng/l and 34ng/l). ACS: Acute Coronary Syndrome, CCD: Chronic cardiac disease, CVA: cerebrovascular disease, PVD: peripheral vascular disease, age>75 with DM or BP. N=number of patients per subgroup (patients can appear in more than one subgroup).

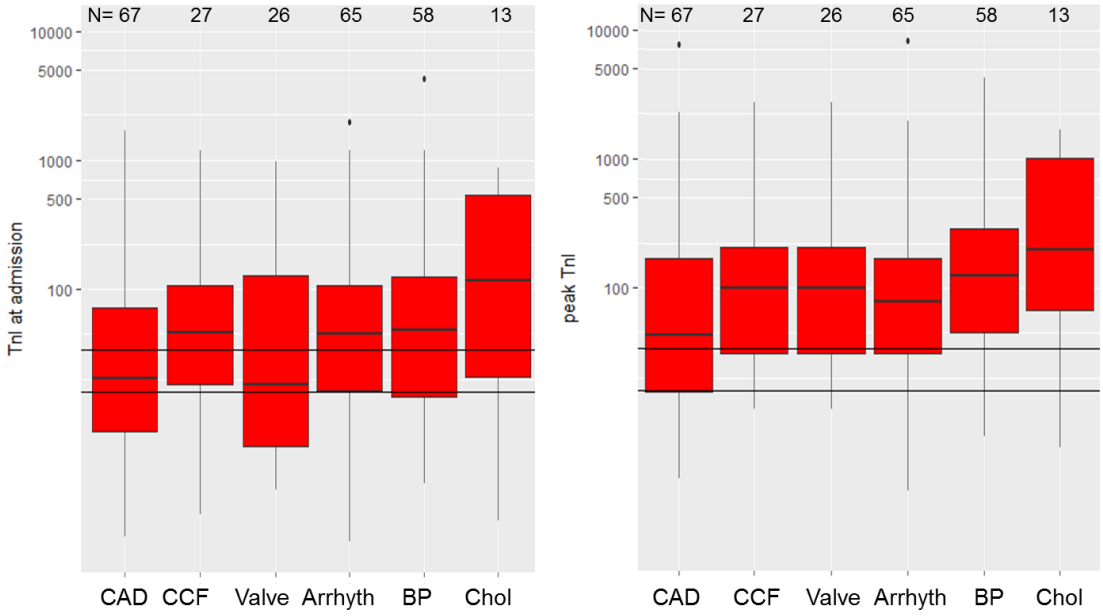

Figure 12: Chronic Cardiac Disease subgroup by TnI (log 10 scale). A: TnI at admission, B: peak TnI. Horizontal lines at sex specific thresholds (16ng/l and 34ng/l). CAD: chronic CAD, CCF: Congestive Cardiac Failure, Valve: valvular disease, Arrhyth: chronic arrhythmia on treatment, BP: hypertensive heart disease, Chol: hypercholesterolaemia heart disease. (Patients can appear in more than one subgroup).

5.4.3.1. Day of peak TnI

The peak TnI for most patients was within 2-3 days of admission to ICU, however there were 74 (27.1%) patients whose peak TnI was between day 4 and day 10 (Figure 13).

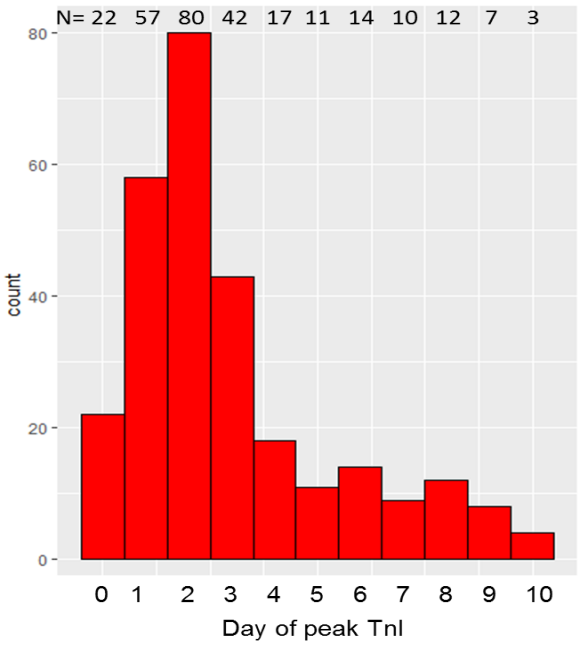

Figure 13: Histogram of day after ICU admission of peak TnI: N= number of patients with peak TnI on each day. Day 0: day before ICU admission, Day 1: day of ICU admission

5.4.3.2. Dynamics of TnI centred around day of peak

Figure 14 shows the dynamics of TnI for all patients, displayed as a fraction of their peak TnI, and centred around the day of peak TnI. It can be seen that patients displayed a dynamic rise and fall pattern, consistent with acute rather than chronic myocardial injury. Overlying Figure 14, and by itself for clarity, is the smoothed conditional mean with 95% confidence intervals. There is a distinctive main peak, surrounded by further, smaller, peaks. Figure 15 shows the dynamic rise and fall pattern visible using the absolute magnitude in TnI (rather than the percentage) across the four quartiles of peak TnI. Even patients with peak TnI below the diagnostic threshold demonstrated a rise and fall pattern. 89% (n=33) of patients who died in ICU also had a dynamic rise and subsequent fall pattern before they died (Figure 16), four patients died on the day of peak TnI.

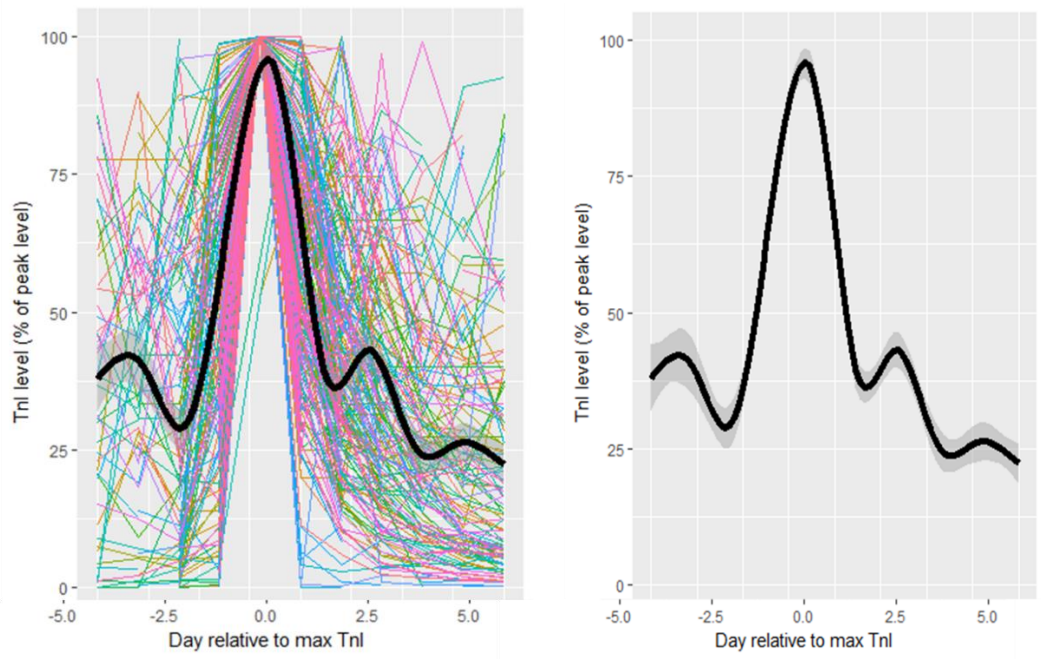

Figure 14: Dynamics of TnI, centred around day of maximum TnI. TnI expressed as a fraction of the peak. Each coloured line represents a separate patient. The black line represents the "average" patient

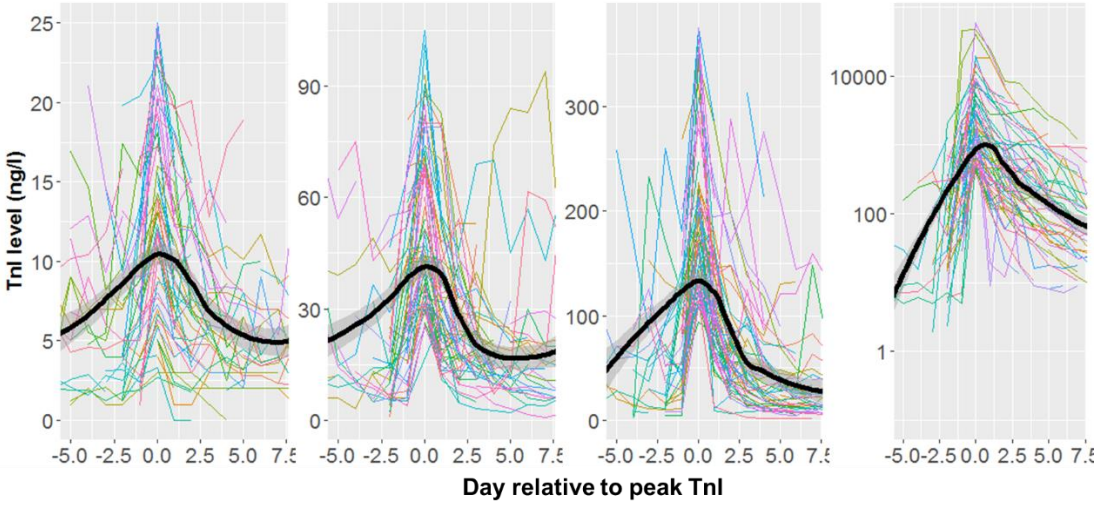

Figure 15: Dynamics of TnI, centred around day of peak TnI. Stratified by quartiles to display dynamic rise and fall pattern throughout the range of peak TnI. Q1 <25ng/l, Q2 25-107ng/l, Q3 107-380ng/l, Q4 >380ng/l (Q4 on logarithmic scale as range 380ng/l to 58860ng/l).

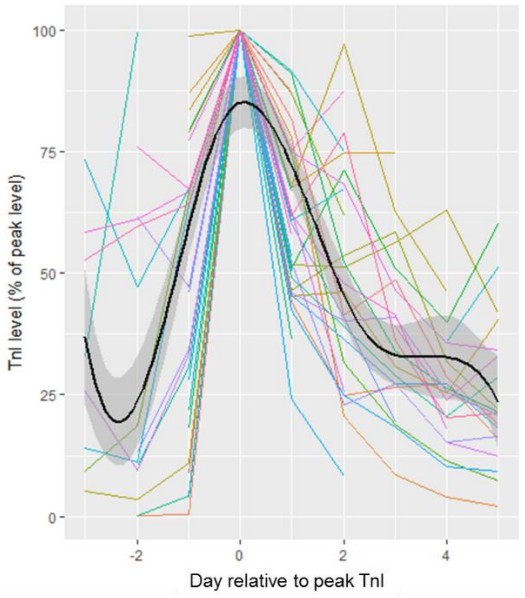

Figure 16: n=37. Dynamics of TnI expressed as fraction of peak, centred around day of peak TnI for patients who died in ICU. 4 patients died on the day of peak TnI (peak TnI 54, 491, 81, 66ng/l). Each coloured line represents a separate patient.

5.4.3.3. Association between Admission TnI and Peak TnI

There was a significant univariable association between admission TnI and peak TnI: an increase in Admission TnI by 1ng/l resulted in an increase of 1.14ng/l (95% CI 0.98 to 1.29) in peak TnI, p<0.001 (Figure 17). This was in part due to the fact that for 57 (20.9%) patients, the admission TnI was also the peak TnI (Figure 13).

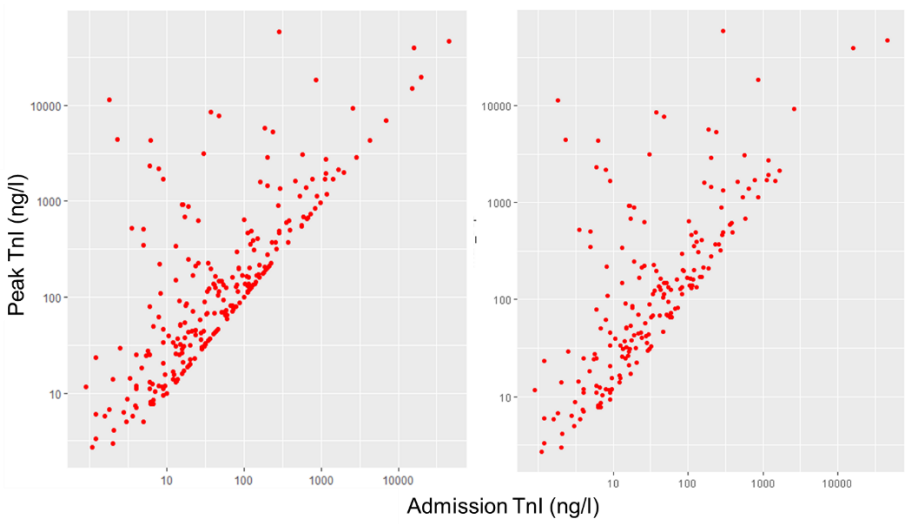

Figure 17: Relationship between Admission TnI (ng/l) and Peak TnI (ng/l). Axes on log10 scale. Left: whole cohort, Right: Exclusion of patients where admission TnI was peak TnI. Univariable association between Peak TnI and Admission TnI (excluding patients where admission TnI = peak TnI): An absolute increase in Admission TnI by 1ng/l resulted in an absolute increase of 1.19ng/l (95% CI 0.98 to 1.39) in peak TnI, p<0.001.

5.4.4. ECG agreement

56 patients' ECGs were analysed by 2 cardiologists independently (Table 20). Reader 2 interpreted more ECGs as abnormal, with considerably more ST depression and T wave inversion. There was fair agreement for whether the baseline ECG was normal or abnormal. There was good agreement (kappa=0.628) for ST elevation

and RBBB (0.698), fair agreement for ST depression (0.249) and only slight agreement for T wave inversion (0.138).

There was excellent agreement as to whether there were dynamic changes consistent with ischaemia when the cardiologists looked at all ECGs for each patient (0.890). Again there was good agreement for dynamic RBBB (0.665), and only fair agreement for dynamic ST depression (0.217) and dynamic T wave inversion (0.223).

Subsequent ECGs were read by one cardiologist.

Table 20: ECG agreement for subgroup of 56 patients. Independent interpretation by Reader 1 and Reader 2. Abnormalities reported by patient (i.e Tw inversion=13 means 13 separate patients with Tw inversion). Kappa statistic for agreement.

	Reader 1	**Reader 2**	**kappa**
n	56	56	
Baseline ECG abnormal y/n	25	38	0.390
ST elevation	7	5	0.628
ST depression	6	11	0.249
Tw inversion	6	16	0.138
Q waves	0	0	-
RBBB	5	6	0.698
Ischaemic changes on ECG (Y)	24	27	0.890
ST elevation	2	0	-
ST depression	2	3	0.217
Tw inversion	6	13	0.223
Q waves	0	1	-
RBBB	1	1	0.665

5.4.5. Objective 1: To determine the incidence of myocardial infarction and myocardial injury as defined by the Third Universal Definition of myocardial infarction.

5.4.5.1. Baseline ECG

255 (92.4%) patients had an ECG taken within six hours of ICU admission. 35.7% (n=91) of these admission ECGs were abnormal with changes associated with potential ischaemia: either ST elevation, ST depression, T wave inversion, Q waves or RBBB (Figure 18, Figure 19). A further 42 were classified as abnormal, but not with changes associated with potential ischaemia (including rhythm disturbances such as atrial fibrillation/flutter and heart block). 274 patients had at least two ECGs enabling assessment of dynamic changes consistent with ischaemia. 27.0% (n=74) of patients had dynamic changes consistent with ischaemia on their ECGs in the first five days of their ICU admission (Table 21, Figure 18). 48.3% of patients who presented with a potentially ischaemic ECG at admission had no dynamic changes (eg persistent ST elevation or ST depression), highlighting the importance of performing serial ECGs in this population. 36.4% of patients who had dynamic ECG changes consistent with ischaemia in the first five days of ICU had no signs of ischaemia on their ICU admission ECG.

Table 21: Potential ischaemia on admission ECG (n=255) and dynamic changes on ECG consistent with ischaemia (n=274), by type of cardiovascular disease. Patients can appear in more than one subgroup. 2 ECGs required for diagnosis of ischaemic changes, 274 patients had at least 2 ECGs.

	n	Potential ischaemia admission ECG	%	n	Dynamic ischaemia on ECG	%
All CVD	255	91	(35.7)	274	74	(27.0)
ACS	20	10	(50.0)		8	(40.0)
CCD	161	66	(41.0)		48	(29.8)
CVA/TIA	34	11	(32.4)		13	(38.2)
PVD	53	20	(37.7)		17	(32.1)
75 DM/BP	85	42	(49.4)		25	(29.4)

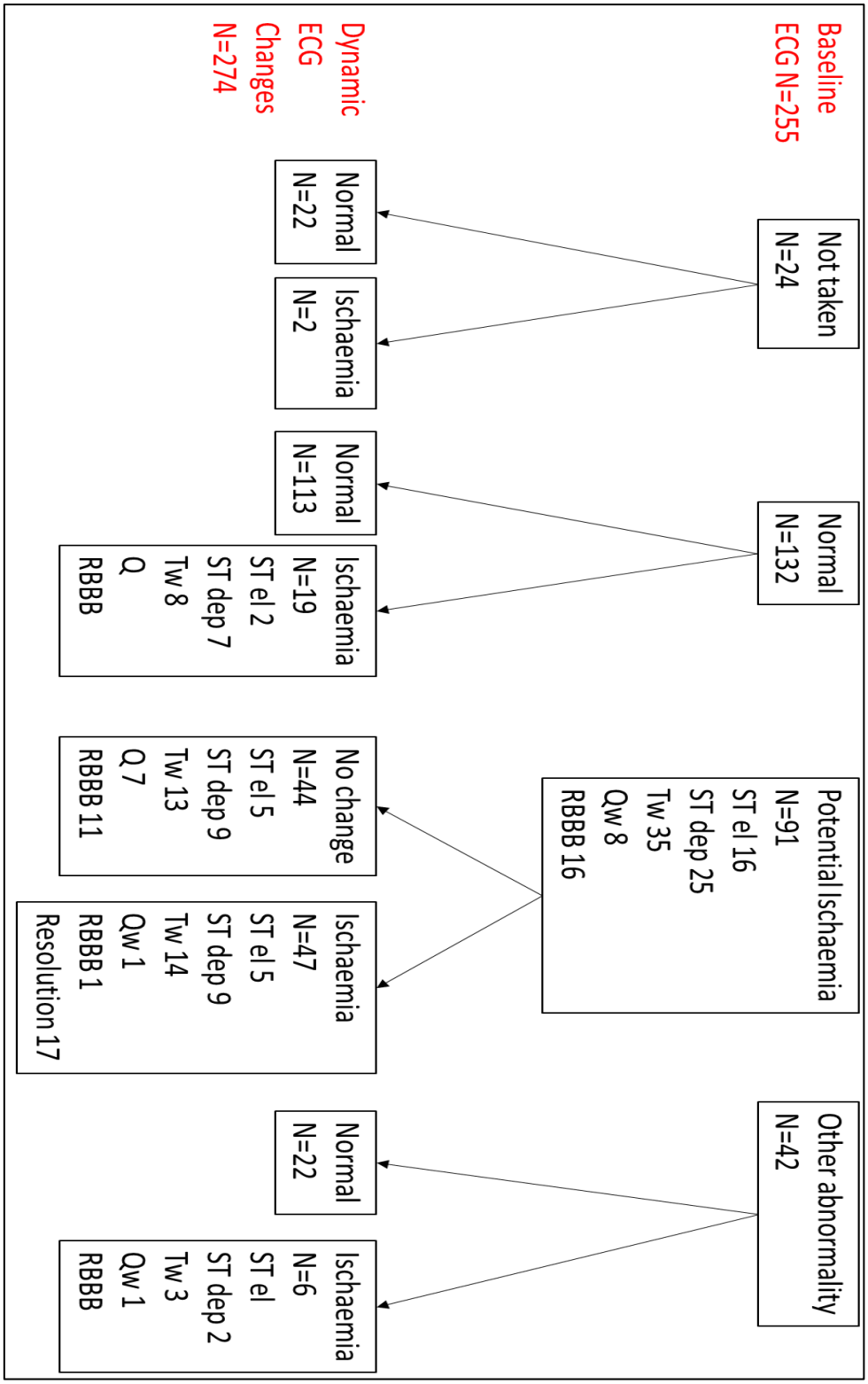

Figure 18: Admission ECG characteristics and dynamic changes consistent with ischaemia.

There was a wide range in admission TnI. For the 142 patients whose admission ECG had no signs of potential ischaemia, the median admission TnI was 28ng/l, below the diagnostic threshold for myocardial infarction (Table 21, Figure 19). Patients who had Q waves or RBBB on their ECG at admission also presented with lower TnI than other ECG abnormalities. With a normal baseline ECG, the median peak TnI was 68ng/l, considerably

lower than for patients who presented with abnormal ECGs (Figure 19). There was a significant univariable association between baseline ECG abnormality and both admission TnI (p<0.001) and peak TnI (p=0.004).

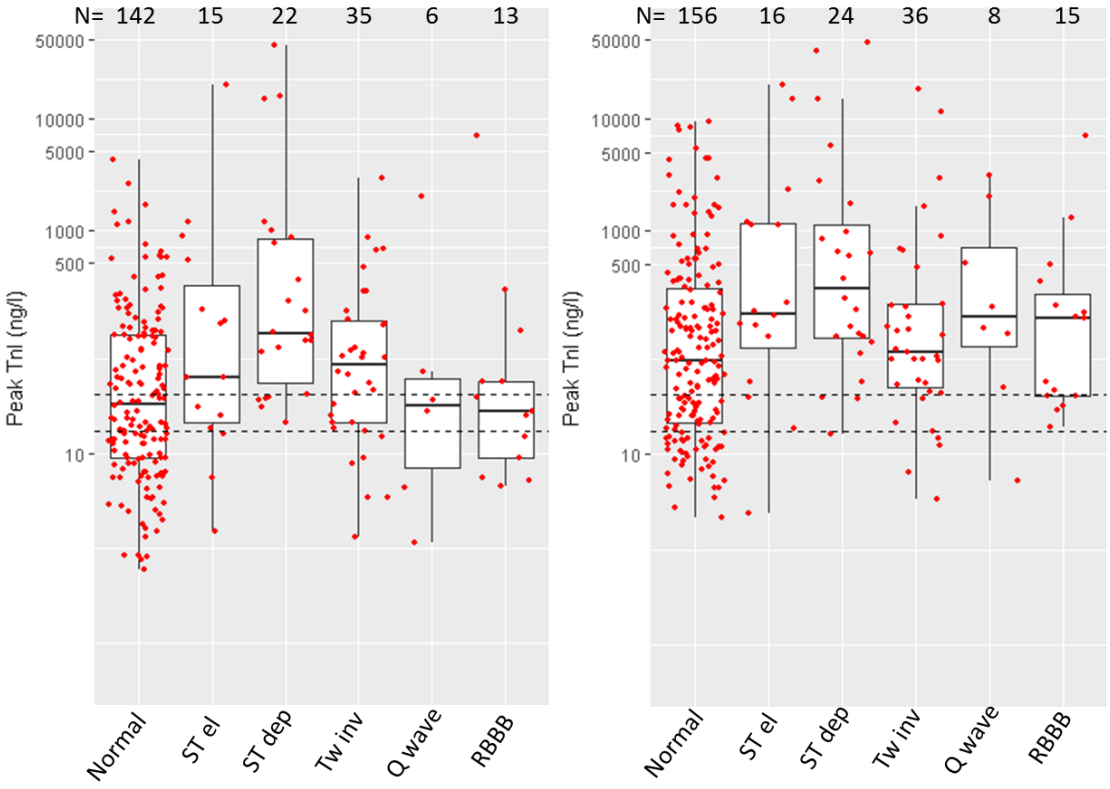

Figure 19: Left: Admission TnI by main Admission ECG (n=233 patients who had admission ECG and admission TnI), Kruskal-Wallis test p<0.001. Right: Peak TnI by main Admission ECG abnormality (n=255 patients who had admission ECG and peak TnI). TnI on log10 scale. Box plots represent median and IQR. Red dots: individual peak TnI. Horizontal dotted lines at TnI=16 & 34ng/l.), Kruskal-Wallis test p=0.004.

5.4.5.2. Dynamic Changes on ECG

274 patients had at least two ECGs, enabling assessment of dynamic changes consistent with ischaemia. 200 patients had no dynamic ECG changes consistent with ischaemia, compared with 74 patients who had dynamic ischaemic changes on ECG. TnI for patients with no ischaemia were significantly lower at admission (med 22ng/l, IQR 9, 102) and peak (med 66ng/l, IQR 18, 202) compared with patients who had dynamic ischaemic changes on ECG (adm TnI med 114, IQR 34, 443; peak TnI 274, IQR 114, 1243), p <0.001. Peak TnI was more strongly associated with dynamic ischaemic changes on ECG than with an admission ECG potentially associated with ischaemia (Figure 20).

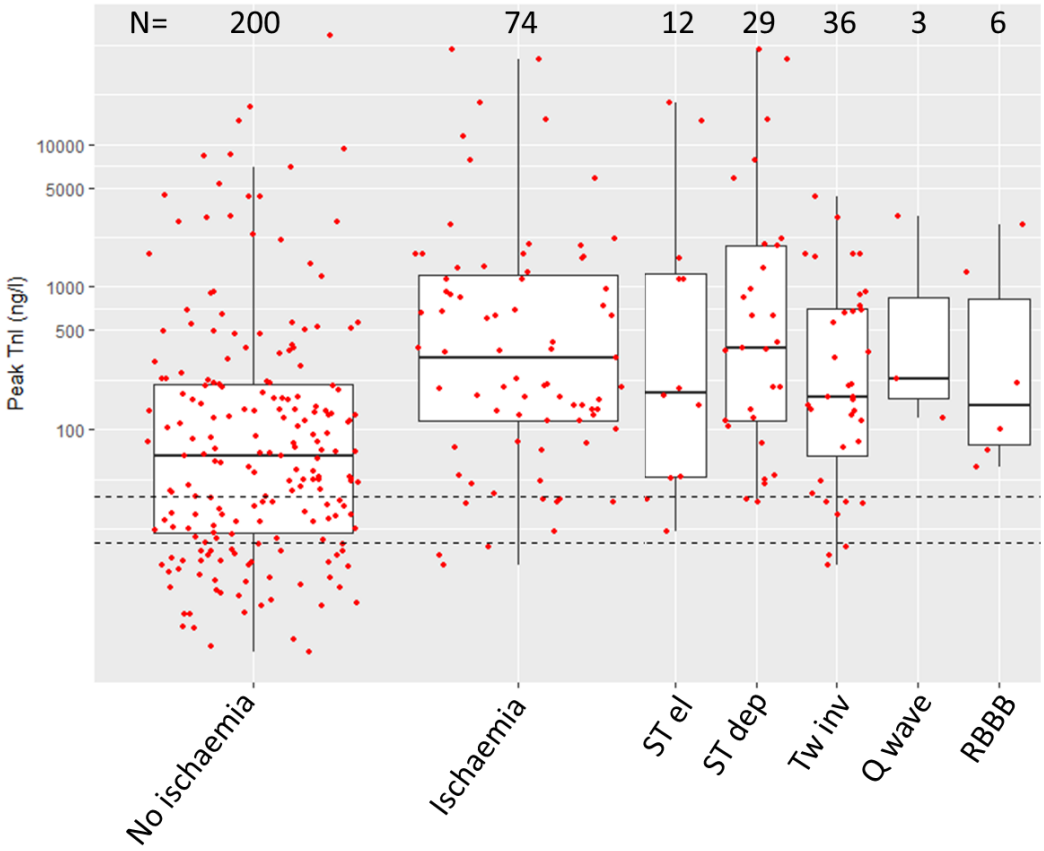

Figure 20: Main Dynamic Change on ECG vs peak TnI. TnI on log10 scale. No ischaemia vs ischaemia. Ischaemia further categorised into type of ECG change. Box plots represent median and IQR. Red dots: individual peak TnI. Horizontal dotted lines at TnI=16 & 34ng/l.

5.4.5.3. Clinically diagnosed cardiac events

There were 12 (4.4%) patients who were diagnosed with myocardial infarction by clinicians who were blinded to the study TnI (Table 22). Of these 12 patients, 11 had peak TnI over the sex-specific diagnostic threshold. Our cardiologists, blinded to TnI, diagnosed ischaemia on only four patients' ECGs (ST elevation n=2, ST depression n=1, T wave inversion n=1). One patient underwent percutaneous coronary angiography and eight patients were started on cardiac medication. Seven patients had a transthoracic echo: three patients had global dysfunction, and two patients had regional wall motion abnormalities detected. 37 patients had clinically documented SVT (including atrial fibrillation and atrial flutter) or VT. 29 of these patients were started on cardiac medication.

*Table 22: Clinically diagnosed cardiac events. *2 patients had cardiac arrest, therefore peak TnI for both patients presented. $TnI 33.6, rounded up to 34, therefore under diagnostic threshold.*

	n	Peak TnI (med)	Q0, Q1, Q3, Q4
Myocardial infarction	12	926	34$^{\$}$, 739, 6968, 47085
Cardiac arrest*	2	169, 4439	
SVT/VT	37	165	2, 44, 680, 6968

5.4.5.4. Classification of Myocardial Injury

76 (27.7%) patients had "No Injury": no TnI rise above the sex-specific diagnostic threshold, and no ischaemic changes on ECG (Figure 21). A further eight patients had no troponin rise, but their ECGs were classified as ischaemic by the cardiologists. Seven of these patients had dynamic T wave inversion. 124 (45.2%) patients were classified as "Injury", with TnI rise above the sex-specific threshold and no ischaemic changes on ECG. 66 (24.0%) of patients were classified as "Infarction", with TnI rise above the sex-specific threshold, and dynamic changes on ECG consistent with ischaemia.

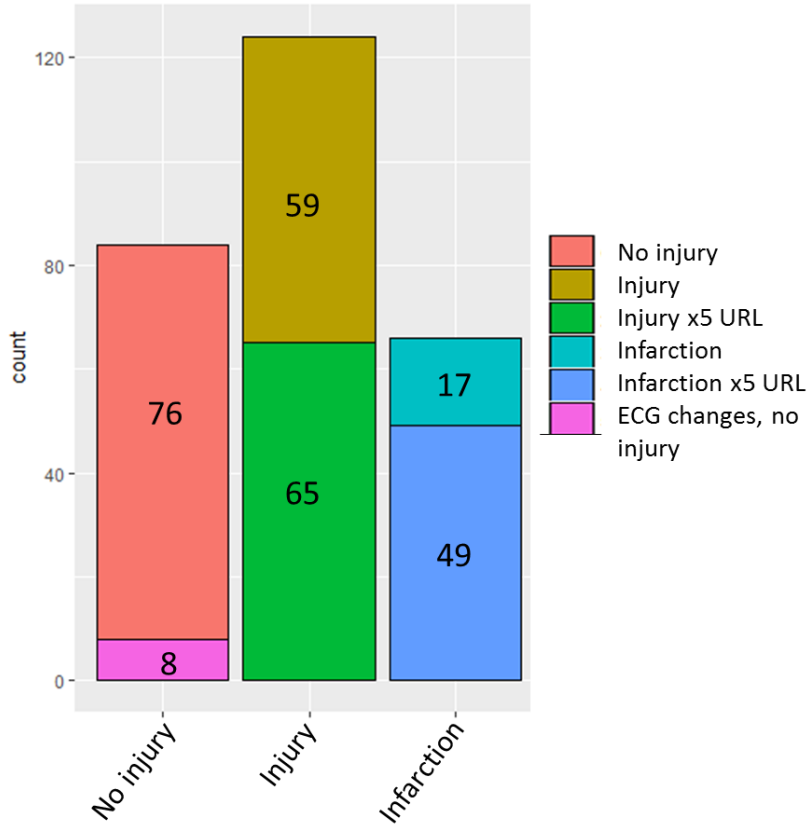

Figure 21: n=274. Number of patients with Myocardial injury categorised by No injury, Injury, Infarction. Injury and Infarction also restricted to > x5 URL

We included the eight patients with ECG changes but no troponin rise into the "No Injury" group, as T wave inversion was non-specific with only slight to fair agreement, and their baseline characteristics and outcomes were very similar.

When we restricted the Injury and Infarction groups to >x5 the sex-specific URL, we found that "No Injury" accounted for 160 patients (58.3%), "Injury" 65 (23.7%), and "Infarction" 49 (17.9%) patients. Patients with Infarction had considerably higher peak TnI (med 370ng/l, IQR 136, 1388) compared with patients with Injury (med 141ng/l, IQR 69, 492) (Table 23). The rise and fall pattern was present throughout all groups, suggesting an acute pattern of injury.

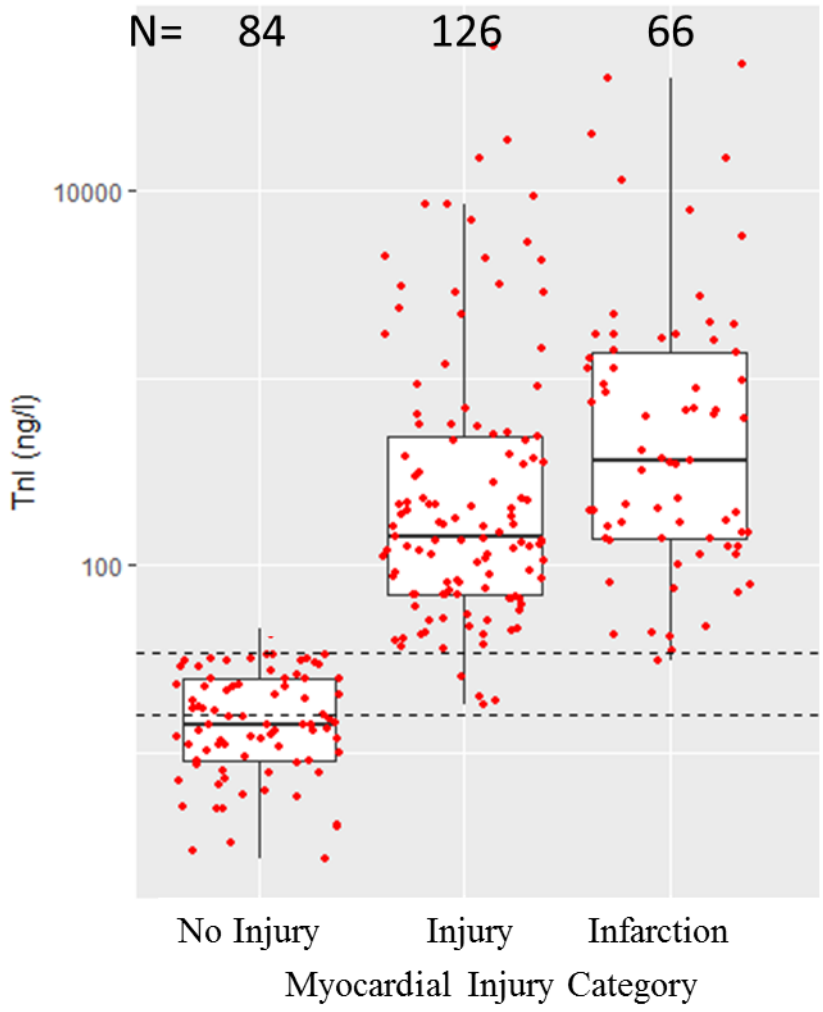

Figure 22: Dynamics of peak TnI by No injury (TnI<threshold); Injury (TnI>threshold, no ECG ischaemia); Infarction (TnI elevation + ECG ischaemia). TnI on log10 scale. Box plots represent median and IQR. Red dots: individual peak TnI. Horizontal dotted line at sex specific thresholds TnI=16 & 34ng/l.

Using our classification, three patients diagnosed with MI by clinicians would have met criteria for Infarction, eight for Injury, and one for No injury. This was the same if the thresholds were restricted to x5 URL. There was clear discordance between clinical diagnosis in routine practice, and diagnosis based strictly on the third universal definition. Myocardial injury was heavily underdiagnosed in clinical practice (12 vs 49 patients), and there was very poor agreement between clinicians and the systematic formal classification, both in terms of positives and negatives.

Table 23: Number of patients with and without ischaemic changes on ECG, in each quartile for TnI.

	Q1 <25ng/l	**Q2 25-107ng/l**	**Q3 107-380ng/l**	**Q4 >380ng/l**
Ischaemia No "Injury"	64	53	43	35
Ischaemia Yes "Infarction"	4	15	25	33

5.4.5.5. Prediction of dynamic ischaemia based on magnitude of TnI

The optimal cutpoint using Youden's index was 0.37 (Figure 23). At this point, the sensitivity for the prediction of ischaemia based on TnI as a continuous variable was 0.69, and the specificity was 0.68. The positive predictive value was 0.46, and the negative predictive value was 0.85, reflecting the low risk of dynamic ischaemia with low TnI values. The c-index for the ROC curve was 0.72 (95% CI 0.66 to 0.79) which was only reasonable discrimination. The magnitude of TnI alone is not a strong discriminator of dynamic changes versus no changes on ECG which might delineate true ischaemia from other mechanisms of injury. It therefore suggests that systematic use of ECG may add information about the mechanism (and by implication possible interventions) for myocardial injury in this setting.

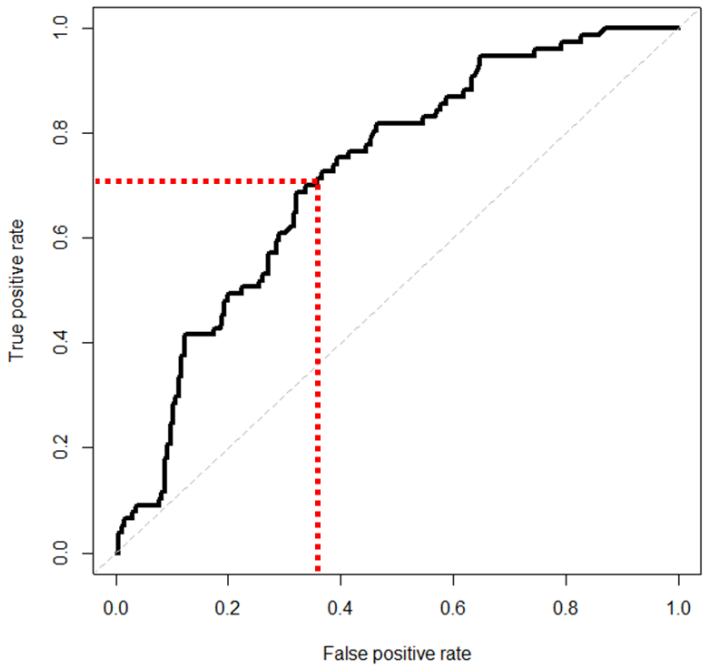

Figure 23: Receiver Operating Characteristic (ROC) Curve for the association of peak TnI with dynamic ischaemic changes on ECG. AUC 0.72, 95% CI 0.66-0.79. Red dotted line: maximum value of Youden's index for ROC curve (optimal cutpoint). At optimal cutpoint: Sensitivity 0.69, Specificity 0.68, PPV 0.46, NPV 0.85.

5.4.5.6. Patient Characteristics stratified by myocardial injury

Patients with Injury and Infarction were older, more likely to be female, to have been admitted as an emergency, and to have higher severity of illness scores in the first 24 hours (Table 24). When we looked at the patients who were recruited as high risk for CVD (age >75years with diabetes or hypertension), but who had no other diagnosed cardiovascular comorbidity, we found that only 7 (15.9%) patients had a peak TnI lower than the diagnostic threshold ("No Injury"). There was no difference in the severe comorbidity count (APACHE) or less severe functional comorbidity index. Presenting diagnoses by system were similar, although genito-urinary disorders were less likely to be in the No Injury group.

*Table 24: Patient characteristics. Stratified by "No Injury" (TnI<sex specific threshold, no ECG changes), "Injury" (TnI>threshold, no dynamic ECG changes), "Infarction" (TnI>threshold, dynamic ischaemic changes on ECG). Patients can appear in more than one CVD subgroup. *Infarction vs Injury for APACHE Score p=0.040*

Variable	No injury	%/ SD/IQR	Injury	%/ SD/IQR	Infarction	%/ SD/IQR	p value
	84	**(30.1)**	**124**	**(44.4)**	**66**	**(23.7)**	
Age mean(SD)	**69.9**	**(10.5)**	**73.9**	**(10.7)**	**74.4**	**(11.0)**	**0.011**
Male sex n(%)	**70**	**(83.3)**	**80**	**(64.5)**	**46**	**(69.7)**	**0.012**
CVD (%)							
ACS	**4**	**4.76**	**10**	**8.06**	**8**	**12.12**	
Chronic cardiac	**56**	**66.67**	**75**	**60.48**	**39**	**59.09**	
CVA/TIA	**15**	**17.86**	**16**	**12.90**	**11**	**16.67**	
PVD	**18**	**21.43**	**10**	**8.06**	**17**	**25.76**	
>75 DM/BP	**16**	**19.05**	**50**	**40.32**	**23**	**34.85**	
Presenting diagnosis							
Resp	**20**	**(30.8)**	**30**	**(46.2)**	**13**	**(20.0)**	
CVS	**19**	**(29.7)**	**28**	**(43.8)**	**17**	**(26.6)**	
GI	**29**	**(34.5)**	**34**	**(40.5)**	**20**	**(23.8)**	
Neuro	**5**	**(41.7)**	**5**	**(41.7)**	**2**	**(16.7)**	
Genito-urinary	**6**	**(19.4)**	**15**	**(48.4)**	**10**	**(32.3)**	
Other	**5**	**(22.7)**	**12**	**(54.5)**	**3**	**(13.6)**	
Comorbidity							**0.507**
Apache: 0	**69**	**(29.6)**	**108**	**(46.4)**	**53**	**(22.7)**	
1	**12**	**(33.3)**	**14**	**(38.9)**	**9**	**(25.0)**	
≥2	**3**	**(30.0)**	**2**	**(20.0)**	**4**	**(40.0)**	
FCI med(IQR)	**2.5**	**(2, 3)**	**2**	**(1, 3)**	**2**	**(1, 4)**	**0.085**
Admission type							**0.013**
Elect Surgical	**26**	**(46.4)**	**22**	**(39.3)**	**7**	**(12.5)**	
Emerg Surgical	**21**	**(25.9)**	**33**	**(40.7)**	**26**	**(32.1)**	
Non-operative	**37**	**(26.1)**	**69**	**(48.6)**	**33**	**(23.2)**	
Severity of Illness							
APII score	**16**	**(13, 18)**	**20**	**(15, 24)**	**20.5**	**(18, 27)**	**<0.001 ***
Vasopressors n(%)	**31**	**(29.8)**	**47**	**(45.2)**	**26**	**(25.0)**	**0.102**
Mech Vent n(%)	**41**	**(30.6)**	**52**	**(38.8)**	**37**	**(27.6)**	**0.165**
RRT n(%)	**1**	**(12.5)**	**6**	**(7.5)**	**1**	**(12.5)**	**0.224**
Hb at admission g/l med (IQR)	**113**	**(100, 130)**	**110**	**(93, 124)**	**107**	**(90, 119)**	**0.064**
nadir Hb	**91**	**(77, 103)**	**84**	**(76, 97)**	**81**	**(74, 95)**	**<0.001**
Admission TnI ng/l med (IQR)	**9**	**(4,15)**	**54**	**(22, 140)**	**152**	**(49, 656)**	**<0.001**
Peak TnI ng/l	**14**	**(9, 23)**	**138**	**(69, 468)**	**375**	**(144, 1376)**	**<0.001**

5.4.5.7. Outcomes stratified by myocardial injury

Patients in the No injury group had significantly lower ICU (p=0.010), hospital (p=0.002) and 6 month (p=0.015) mortality than patients with Injury or Infarction (Table 25). Patients with Infarction had similar ICU (p=0.930), hospital (p=0.163) and 6 month (p=0.420) mortality to those with Injury. Patients with Infarction had longer duration of mechanical ventilation than patients with Injury (p=0.001). ICU and hospital length of stays were similar across the Injury and Infarction groups.

Table 25: Outcomes stratified by myocardial injury. Categorical n(%) p value Chi^2 test, continuous median(Q^0,Q^1,Q^3,Q^4). p value Injury vs Infarction = Kruskal-Wallis

	No injury	**%**	**Injury**	**%**	**Infarction**	**%**	**P** Injury vs Infarction
N	84		126		66		
ICU mortality	4	(4.8)	22	(17.5)	11	(16.7)	0.930
Hospital mortality	8	(9.5)	30	(23.8)	21	(31.8)	0.163
6 month mortality	14	(16.7)	39	(31.0)	24	(36.4)	0.420
MV duration	1	(0, 0, 4, 33)	2	(0, 0, 5, 31)	4	(0, 2, 7, 39)	0.001
ICU LOS	5	(0, 3, 9, 53)	7	(0, 4, 10, 36)	7	(0, 4, 13, 43)	0.380
Hospital LOS	17	(3, 11, 26, 125)	18	(1, 10, 31, 157)	21	(2, 11, 39, 118)	0.318

5.4.5.8. Summary: myocardial injury category

Using a systematic categorisation in line with the Third Universal Definition for Myocardial Infarction, we classified 84 (30.7%) of our patients into the No Injury group, 124 (45.3) patients into the Injury group, and 66 (24.1%) patients into the Infarction group. This had little agreement with Infarction diagnosed by clinicians. The use of ischaemia rather than specific ECG abnormalities resulted in a highly reliable assessment of the ECG. 48.3% of patients who presented with a potentially ischaemic ECG at admission had no dynamic changes, highlighting the importance of performing serial ECGs in this population. 36.4% of patients who had dynamic ECG changes consistent with ischaemia in the first five days of ICU had no signs of ischaemia on their ICU admission ECG, showing the importance of continued ECG surveillance during acute critical illness.

Patients with Infarction had significantly higher peak TnI compared with the Injury group. However, TnI value was only a moderate discriminator between the injury and infarction group once dynamic ECG changes were considered. Patients with Injury and Infarction were older and sicker at presentation to ICU than patients with No Injury, and had a longer duration of mechanical ventilation and hospital stay, and lower long term survival. There was no statistically significant difference in outcomes between Injury and Infarction groups in this univariable analysis based on the number of cases available.

5.4.6. Objective 2: To explore the duration of TnI elevation above baseline with respect to mechanism of injury (cardiomyocyte necrosis vs reversible ischaemia).

The median time for TnI to fall to 25% of its peak value, or below the sex-specific diagnostic threshold was 3 days (IQR 2,4) (Figure 24). Examination of patients where TnI fell below threshold by day 1 (n=32) compared

with those where TnI fell below threshold on or after day 4 (n=51) showed no difference in type of admission, type of cardiovascular disease, presence of ischaemia on ECG, severity of illness on the day of peak TnI, or use of inotropes or mechanical ventilation. Patients with longer duration of TnI elevation were sicker at presentation to ICU (APACHE II Score 18 vs 21, p=0.011), and had higher mortality by six months (15.6% vs 37.3%, p=0.047). There was no difference in the duration of TnI elevation between the Injury (med 3, IQR 2, 3) and Infarction (med 3, IQR 2, 4, p=0.057) groups (Figure 25, Figure 26). Approximately 90% of patients in both Injury and Infarction groups returned to baseline within four days of the peak TnI (Figure 26).

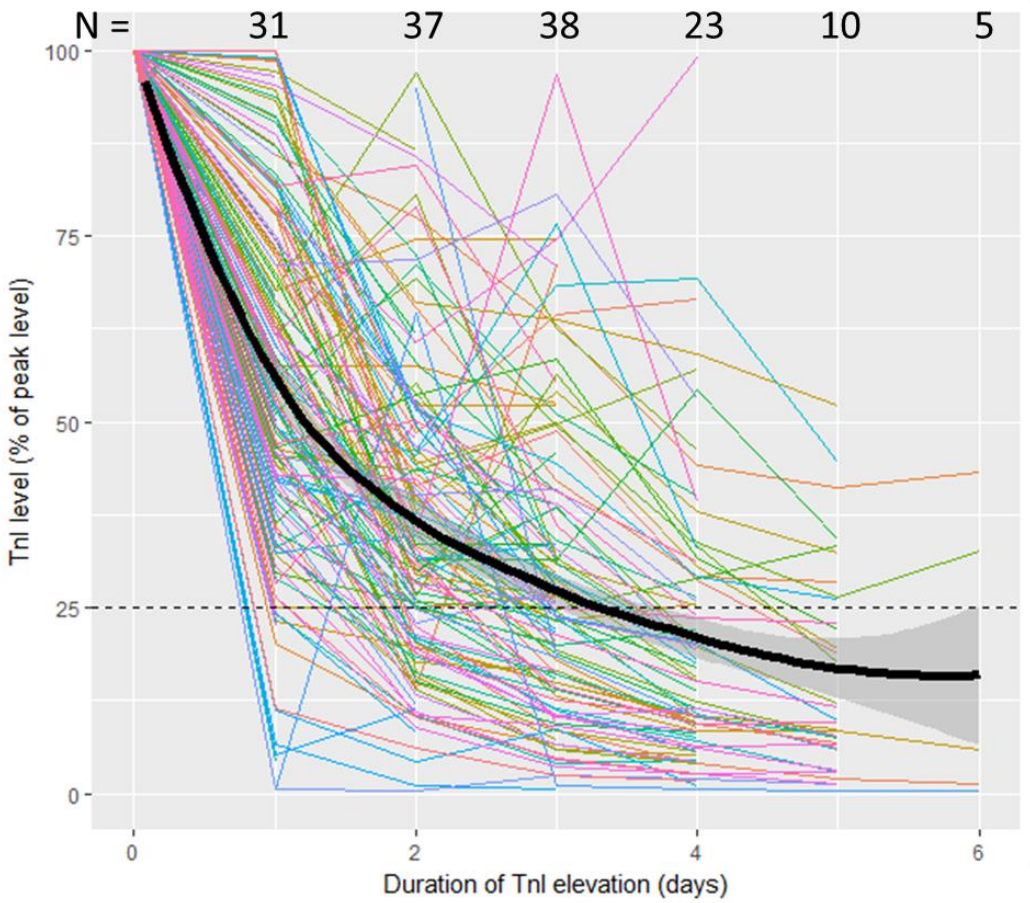

Figure 24: Duration of TnI elevation (days) after peak TnI (% of peak TnI). Restricted to patients who had peak TnI greater than sex-specific threshold (n=176). N=number of patients where TnI dropped below 25% of peak TnI (horizontal dotted line), or below sex-specific threshold. 21 patients TnI threshold not reached and TnI was censored before day 5. 11 patients where TnI collection was censored after 5 days, and TnI threshold not reached.

Table 26: Table of patients where TnI dropped below threshold by day 1 after peak TnI, compared with patients where TnI dropped on or after day 4 after peak TnI. Threshold was defined as 25% of peak value, or below sex-specific diagnostic threshold (34ng/l for men, 16ng/l for women).

	Day 1	**% or IQR**	**Day 4-10**	**% or IQR**	**p**
N	32		51		
Peak TnI (median [IQR])	100	(43, 634)	172	(102, 481)	0.34
Sex (%)	5	(15.6)	18	(35.3)	0.051
Admission type (%)					
El Surg	4	(12.5)	4	(7.8)	0.764
Em Surg	10	(31.3)	18	(35.3)	
Em Med	18	(56.3)	29	(56.9)	
Ischaemia (%)	11	(34.4)	23	(45.1)	0.367
Age (mean (sd))	70.2	(12.5)	73.6	(11.7)	0.218
FCI (median [IQR])	2	(1, 3)	3	(2, 4)	0.004
SOFA (median [IQR])	7	(3, 10)	8	(6, 10)	0.291
APACHE II score (med[IQR])	18	(15, 23)	21	(19, 25)	0.011
Hb (median [IQR])	100	(88, 119)	105	(89, 116)	0.91
Inotropes (%)	16	(50.0)	34	(66.7)	0.168
Mech vent (%)	16	(50.0)	29	(56.9)	0.652
Duration MV (median [IQR])	4	(0, 6)	4	(0, 9)	0.789
ICU mortality (%)	2	(6.3)	12	(23.5)	0.068
Hospital mortality (%)	4	(12.5)	16	(31.4)	0.066
6 month mortality (%)	5	(15.6)	19	(37.3)	0.047

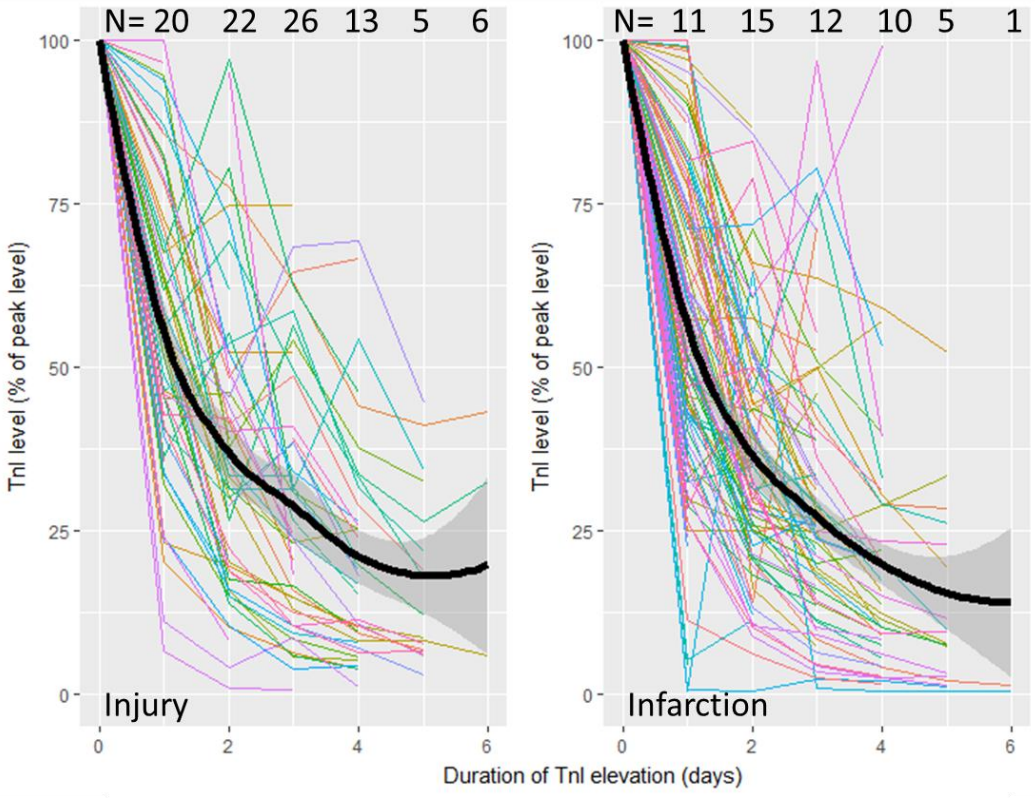

Figure 25: Duration of TnI elevation (days) after peak TnI (% of peak TnI). Restricted to "Injury" and "Infarction" groups. N=number of patients where TnI dropped below 25% of peak TnI (horizontal dotted line), or below sex-specific threshold. Patients where TnI did not fall below threshold before data collection stopped: Injury n=21, Infarction n=11

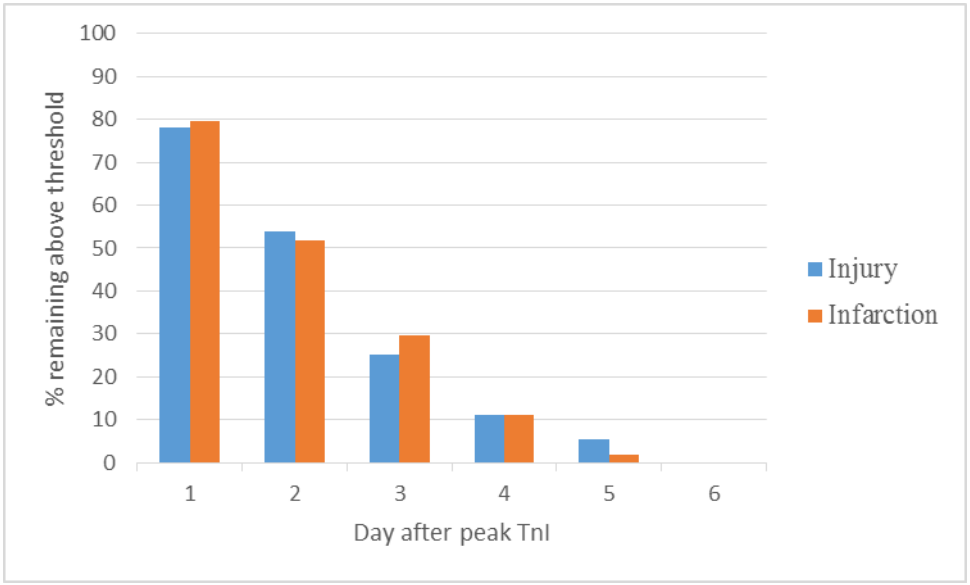

Figure 26: Percentage of patients with TnI above 25% of peak TnI or sex-specific threshold by day after peak TnI. Stratified by Injury vs Infarction.

5.4.7. Objective 3: To explore the relationship between TnI and biomarkers representing global inflammation (C-Reactive Protein, CRP).

Hypothesis: if TnI is released as part of a global inflammatory response, then there will be a temporal relationship between TnI and CRP.

117 patients from seven hospitals had CRP routinely measured from the same sample as TnI (Table 27). These patients were broadly representative of the whole cohort, with mean age 73.9 years, 65% male, and patients recruited mainly due to chronic cardiac disease (40.5%) or >75 with DM/BP (30.2%). Patients were most frequently admitted with Respiratory (33.3%) or gastrointestinal diagnoses (27.4%), and only 12.8% of admissions were post elective surgery. APACHE II scores were similar (med 18, IQR 15, 22), and 31.0% of patients required vasopressor support, and 37.5% of patients were mechanically ventilated during the first 24 hours of ICU admission. 18.8% of patients had dynamic changes on ECG consistent with ischaemia, and admission TnI and peak TnI were similar to the overall cohort.

Table 27: Baseline Characteristics for CRP cohort, compared with overall cohort

Variable	Overall cohort	%/IQR	CRP subgroup	%/IQR
n	279		117	
Age mean(SD)	72.3	(10.9)	73.92	(10.39)
Male sex n(%)	200	(71.7)	76	(65.0)
CVD n(%)				
ACS	22	(7.9)	5	(4.3)
Chronic cardiac disease	174	(62.4)	79	(67.5)
CVA/TIA	42	(15.1)	22	(18.8)
PVD	55	(19.7)	16	(13.7)
>75, DM/BP	90	(32.5)	35	(29.9)
System diagnosis n(%)				
Respiratory	65	(23.3)	39	(33.3)
CVS	64	(22.9)	15	(12.8)
GI	84	(30.1)	32	(27.4)
Neuro	12	(4.3)	4	(3.4)
Genito-urinary	31	(11.1)	15	(12.8)
other	22	(7.9)	12	(3.4)
Comorbidity				
Apache: 0	233	(83.5)	93	(79.5)
1	36	(12.9)	18	(15.4)
≥2	10	(3.6)	6	(5.1)
FCI med(IQR)	2	(0,1,3,9)	2	(1, 3)
Type of admission n(%)				
Elect Surgical	56	(20.1)	15	(12.8)
Emerg Surgical	81	(29)	28	(23.9)
Non-operative	142	(50.9)	74	(63.2)
ECG ischaemia n(%)	78	(28)	22	(18.8)
Severity of Illness in first 24h				
APII score	18	(15,23)	18	(15, 22)
SOFA score	7	(5,10)	5	(3, 7)
Vasopressors n(%)	104	(37.3)	35	(31.0)
Mech Vent n(%)	134	(48.2)	42	(37.5)
RRT n(%)	8	(2.9)	4	(3.6)
Admission Hb g/l med (IQR)	111	(93, 125)	110	(96, 123)
Admission TnI ng/l med (IQR)	26	(56, 2157)	39	(15, 139)
Peak TnI	105	(375, 58820)	90	(25, 299)
Peak Lactate	2.6	(1.7, 4.2)	2.3	(1.5, 3.3)

There was a wide range of peak CRP (Figure 27), median peak CRP was 254mg/l (IQR 183, 323). There were only three (2.6%) patients who had a peak CRP within normal limits (<10mg/l), and only five (4.3%) who had a peak CRP<50mg/l. The most common day of peak CRP was day 2 of ICU admission (Figure 28). 70% (n=82) of peak CRP were within 5 days of admission to ICU. The rate of increase of CRP (Figure 29) was slower than the rate of increase of TnI (Figure 14). 61% of peak CRP (n=71) occurred within +/- 1 day of the peak in TnI

(Figure 30). There was no association between peak CRP and peak TnI (p=0.879) (Figure 31), or between peak CRP and myocardial injury category (p=0.585) (Figure 32).

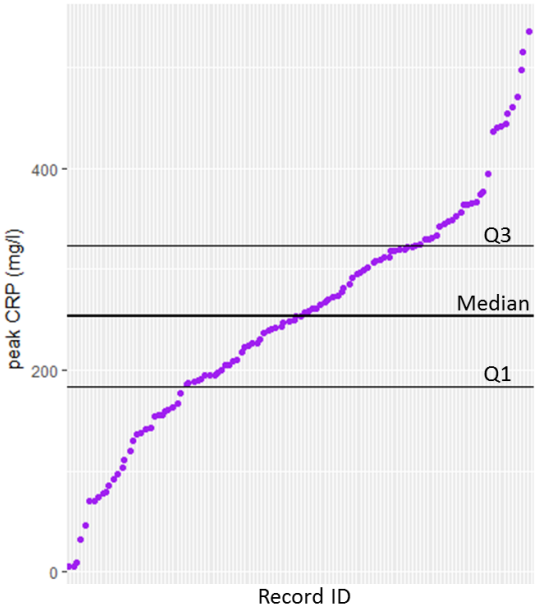

Figure 27: Peak CRP (mg/l) for each patient (n=117). Median peak CRP 254 (Min 5, Q1 183, Q3 323, Max 536). Plotted in ascending order of CRP

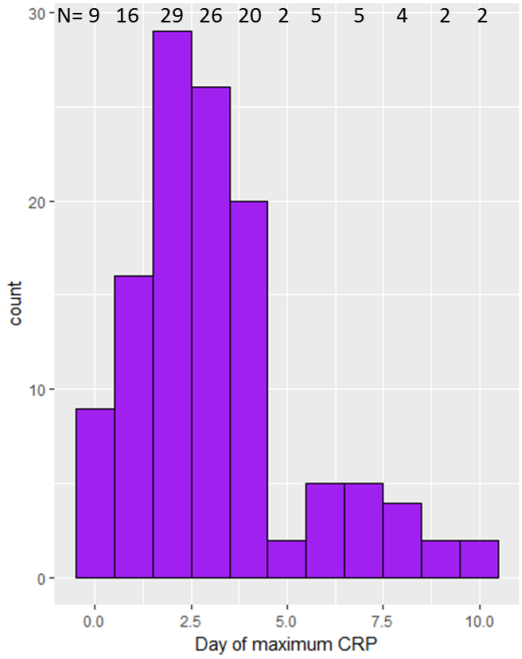

Figure 28: Day of maximum CRP (Day 0 = pre-ICU)

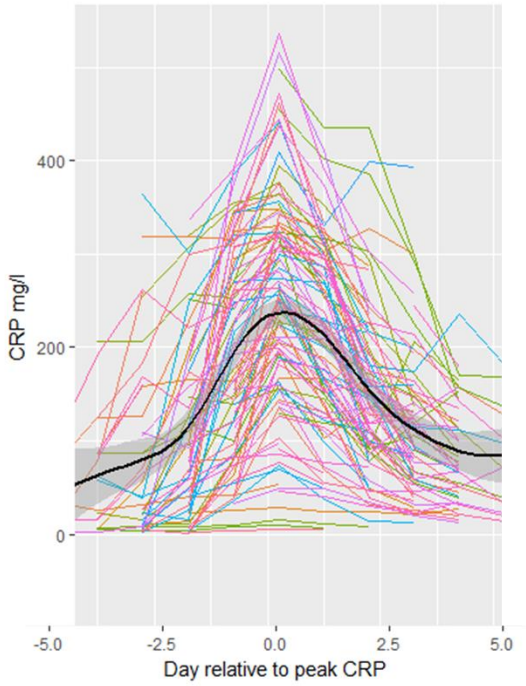

Figure 29: Dynamics of CRP (mg/l) during first 5 days of ICU admission, centred around the day of peak CRP.

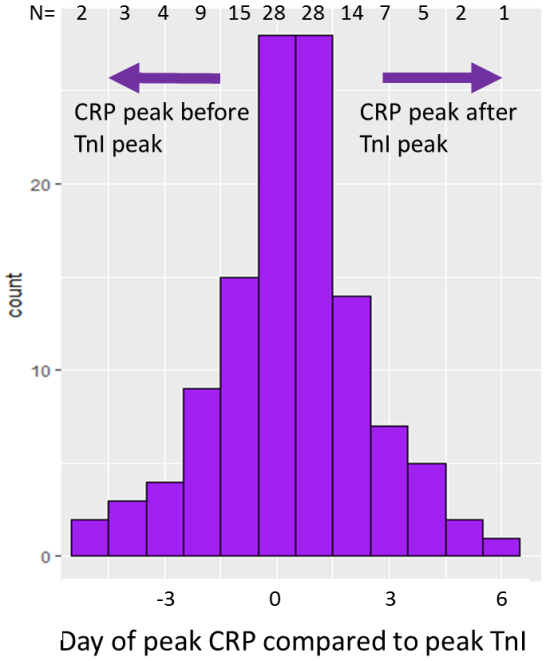

Figure 30: n=117. Time between peak TnI and peak CRP (days). Restricted to first 5 days of ICU admission. "0": peak TnI and CRP from same sample. Negative: CRP peak occurred before TnI peak, Positive: CRP peak occurred after TnI peak.

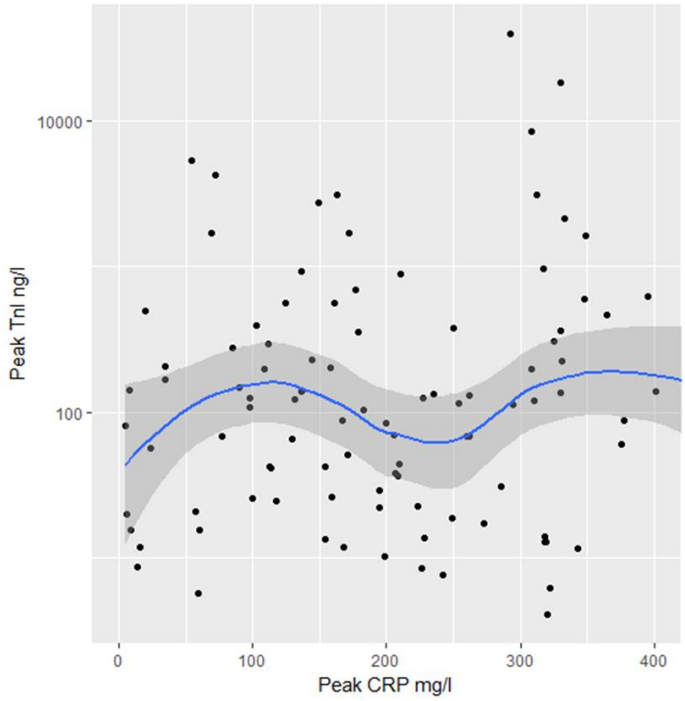

Figure 31: Relationship between Peak CRP (mg/l) and Peak TnI ((ng/l, logarithmic scale). Time restricted to day of peak TnI and day after peak TnI. p=0.879

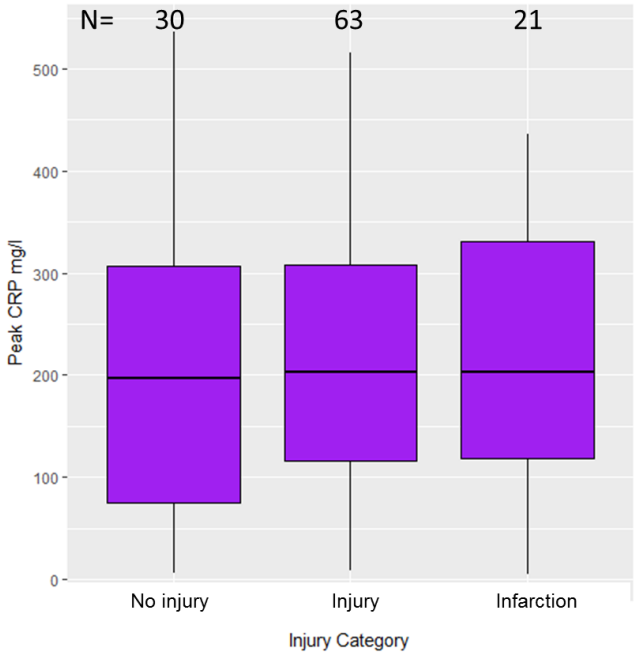

Figure 32: Peak CRP (mg/l) stratified by Myocardial Injury Category. Restricted to 48 hours following peak TnI. Kruskal-Wallis test p=0.585. Spearman's correlation r=0.05.

5.4.7.1. Summary: Relationship of TnI with inflammation (CRP)

CRP was almost always increased in this population during ICU stay. The patterns followed a rise and fall with varying peak value, which most commonly occurred on days 2-3. When we explored the association between TnI peak and CRP peak, we found no apparent association, even after making adjustment for the slower rate of increase of CRP compared to TnI based on their known kinetics following an inflammatory insult or myocardial

ischemia. This lack of association was observed when exploring both the timing of peak value and the maximum value measured for each biomarker. Importantly, there was also no difference in the CRP concentration between the three myocardial injury groupings (no injury, injury, infarction). This does not support the hypothesis that CRP will be associated with TnI if an inflammatory response is the main driver of Tn elevation in this population.

5.4.8. To explore the relationship between TnI and biomarkers representing global ischaemia (lactate).

Hypothesis: If TnI release is as a result of ischaemia, then there will be a temporal relationship between TnI and lactate.

All patients in the TROPICCAL cohort had lactate taken as part of their routine clinical care. The median peak lactate was 2.6 mmol/l (IQR 1.7, 4.2) (Figure 33). The most common day of peak lactate was day 1 of ICU admission (Figure 34). 74.0% (n=202) of peak lactate were within 3 days of admission to ICU. The rate of increase of lactate (Figure 35) was difficult to ascertain as the peak lactate was often the first sample taken.

There were 69 (25.0%) who had at least one lactate measurement >4.0 mmol/l during the first five days after their ICU admission. These patients were similar in age, sex, and comorbidity to the overall cohort (Table 28). A considerably higher proportion of the "High" group presented with cardiovascular diagnoses, and as emergency surgical admissions compared to patients in the "middle" and "low" groups. Patients with high lactates were sicker at presentation to ICU (median APACHE II score 20 IQR (16, 27)), and were more likely to be on vasopressors and receiving mechanical ventilation.

Table 28: Baseline Characteristics of Lactate Subgroups. "High" >4 mmol/l, "Middle "2-4mmol/l, "Low" <2 mmol/l.. Patients with CVD can appear in more than one subgroup.

Variable	High	%	Middle	%	Low	%	p
N	69	25.7	96	35.8	103	38.4	
Age mean(SD)	73.5	(9.3)	73.1	(11.0)	72.2	(11.5)	0.712
Male sex n(%)	51	(73.9)	73	(76.0)	67	(65.0)	0.197
CVD n(%)							
ACS	10	(14.3)	9	(9.5)	3	(3.4)	
Chronic Cardiac Disease	46	(65.7)	60	(63.2)	65	(73.9)	
CVA/TIA	8	(11.4)	20	(21.1)	13	(14.8)	
PVD	19	(27.1)	19	(20.0)	17	(19.3)	
>75 DM/BP	21	(30.0)	35	(36.8)	31	(35.2)	
System Diagnosis							
Respiratory	10	(14.7)	25	(26.0)	28	(27.2)	0.187
CVS	25	(36.8)	21	(21.9)	15	(14.6)	
GI	22	(32.4)	28	(29.2)	31	(30.1)	
Neuro	2	(2.9)	3	(3.1)	7	(6.8)	
Genito-urinary	5	(7.4)	10	(10.4)	15	(14.6)	
Other	4	(2.9)	9		7	(1.9)	
Comorbidity							
Apache: 0	58	(84.1)	82	(85.4)	84	(81.6)	0.483
1	10	(14.5)	9	(9.4)	16	(15.5)	
≥2	1	(1.4)	5	(5.2)	3	(2.9)	
FCI med(IQR)	2	(1, 3)	2	(1, 3)	2	(1, 4)	0.092
Admission Type							
Elect Surgical	10	(14.5)	25	(26)	19	(18.4)	0.019
Emerg Surgical	30	(43.5)	24	(25)	24	(23.3)	
Non-operative	29	(42)	47	(49)	60	(58.3)	
Severity of Illness							
APII score	20	(16, 26)	8	(15, 22)	18	(15, 21)	0.031
Vasopressors n(%)	31	(46.3)	40	(43)	30	(29.4)	0.048
Mech Vent n(%)	40	(59.7)	46	(48.9)	43	(42.2)	0.083
RRT n(%)	3	(4.6)	1	(1.1)	4	(3.9)	0.366
Peak TnI ng/l	321	(54, 1280)	91	(32, 242)	46	(13, 155)	<0.001
Peak CRP mg/l	281	(198, 344)	251	(191, 323)	236.4	(155, 319)	0.363
Peak Lactate (mmol/l)	5.9	(4.5, 8.5)	2.8	(2.4, 3.3)	1.5	(1.2, 1.8)	<0.001
ECG ischaemia n(%)	27	(39.1)	26	(27.1)	21	(20.4)	0.026
Outcomes							
ICU mortality m(%)	15	(21.7)	16	(16.8)	6	(5.9)	0.008
Hospital mortality (n(%)	22	(31.9)	24	(25.3)	13	(12.9)	0.009
6month mortality n(%)	24	(34.8)	34	(35.4)	19	(18.6)	0.015

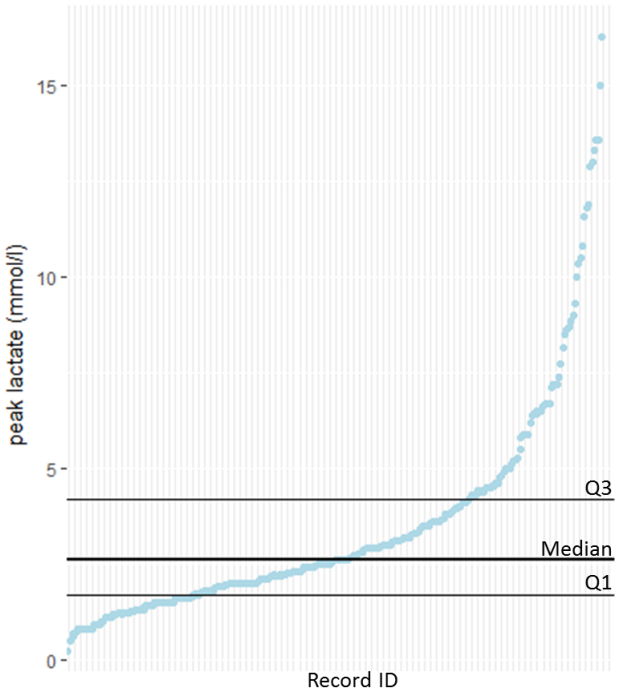

Figure 33: Peak lactate for each patient (mmol/l). Median peak lactate 2.6 (Min 0.2, Q1 1.7, Q3 4.2, Max 16.3). Plotted in ascending order of lactate

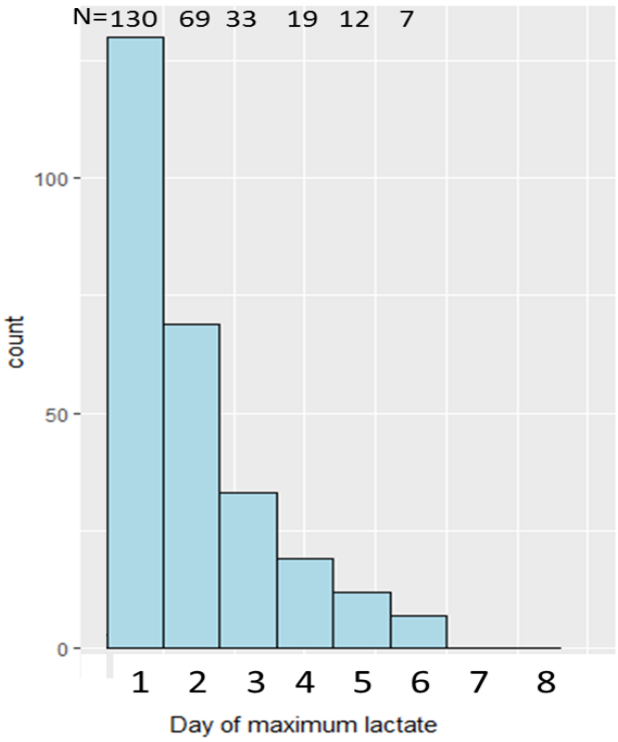

Figure 34: Day of maximum lactate Day 1 = maximum lactate recorded in first 24 hours of ICU admission. N=number of patients with maximum lactate of each day

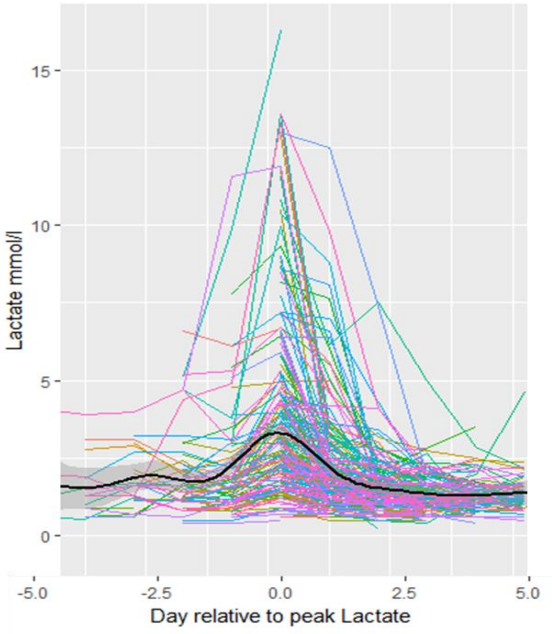

Figure 35: Dynamics of Lactate (mmol/l) during first 5 days of ICU admission, centred around the day of maximum Lactate. As seen in Figure 34, the majority of peak lactate was often the first sample taken, therefore minimal datapoints before this.

Relationship of lactate to peak TnI (Figure 35) shows that there was a dynamic rise and fall pattern seen in lactate. The peak lactate happened in the majority of patients (n=130) in the first 24 hours of ICU (Figure 34), and for many patients, this was their first lactate measurement (Figure 35). The peak in lactate was at broadly similar times to the peak in TnI (Figure 36). 61% (n=164) of peak lactates occurred within +/- 1 day of the peak in TnI. Peak lactate was significantly higher in the groups preceding TnI peak compared with the group post TnI peak (p<0.001), however, there was no difference in peak lactate between the groups >48 hours preceding peak TnI and <48 hours (where there could potentially be a temporal relationship) (p=0.918) (Figure 37).

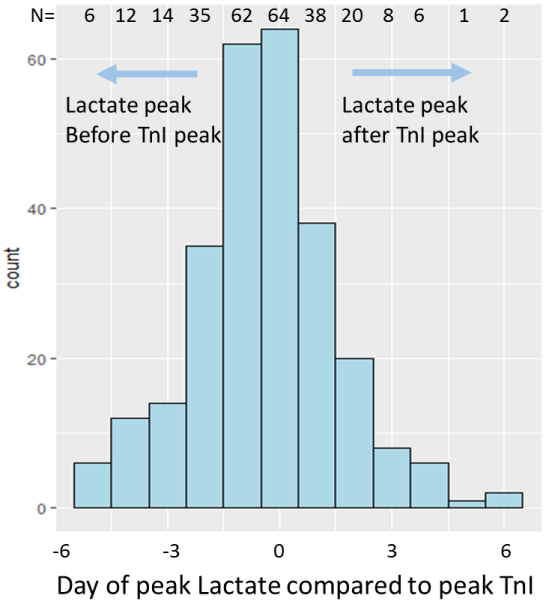

Figure 36: n=268. Time between peak TnI and peak Lactate (days). Restricted to first 5 days of ICU admission. "0": lactate peak in 24 hours preceding TnI peak. Negative: Lactate peak occurred before TnI peak, Positive: Lactate peak occurred after TnI peak.

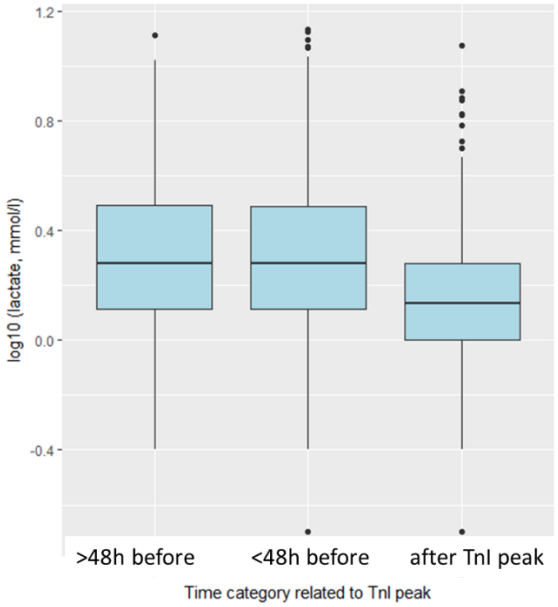

Figure 37: Distribution of lactate relative to day of peak TnI. ">48h before peak TnI; <48h before peak TnI (potentially temporal relationship); All time after TnI peak. One-way repeated measures (Group ">48h" vs"<48h" vs "after") ANOVA p<0.001. Group ">48h" vs Group "<48h" p=0.918.

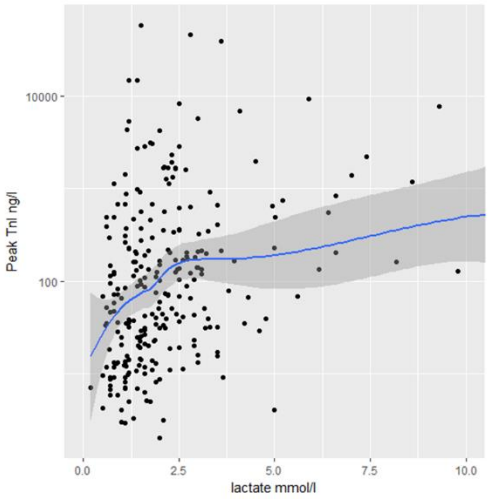

Figure 38: Restriction to 24 hours preceding peak TnI. Scatterplot displaying the relationship between lactate (mmol/l) and Peak TnI (ng/l, logarithmic scale). p=0.0005. Spearman's correlation r=0.33

5.4.8.1. Relationship of lactate to Myocardial Injury category

There was a significant association between peak TnI and lactate in the 24 hours preceding the peak (p=0.0005, Figure 38). When combined with ECG data, the lactate in the 24 hours preceding the peak TnI was significantly lower in the No Injury group (med 1.4mmol/l, IQR 1.1 to 2.0), than in the Injury group (med 1.5, IQR 1.1 to 2.3) and Infarction group (med 1.7, IQR 1.1 to 2.5), p<0.001, Figure 39). 40 (59.7%) patients classified as Infarction had a lactate>2.0 in the preceding 24 hours.

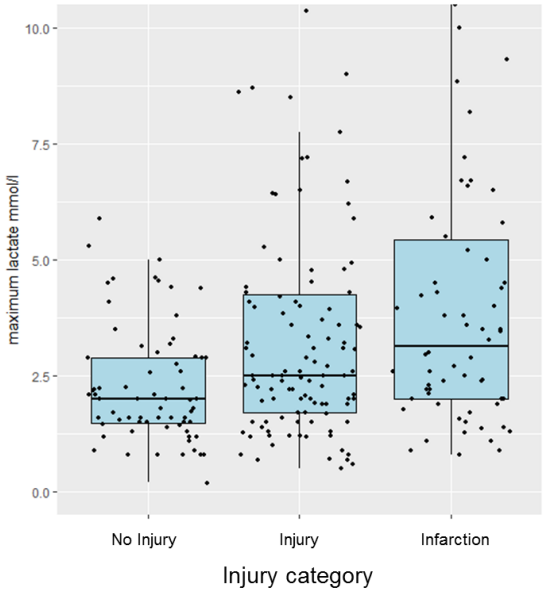

Figure 39: Relationship between Myocardial Injury and peak lactate (mmol/l) in 24 hours preceding peak TnI. 0: No Injury, 1: Injury, 2: Infarction. Black dots represent individual patient maximum lactate values. P<0.001.

5.4.8.2. Summary:

61.5% of patients had a peak lactate >2.0mmol/l. The highest value was most commonly taken in the first 24 hours of ICU admission. After adjustment for temporal relationships, we found a significant association between TnI peak and lactate level in the preceding 24 hours. Furthermore, patients with Infarction had significantly higher lactate levels in the 24 hours preceding peak TnI compared to patients with Injury or No Injury. This supports the hypothesis that Infarction is a distinct category of myocardial injury, and potentially is more attributable to ischaemic mechanisms than inflammatory mechanisms.

5.4.9. Objective 4: To understand the incidence of significant anaemia and its management in critically ill patients with cardiovascular disease, and its relationship with TnI

There was no significant differences in age, sex, comorbidity or admission type at presentation for those patients who developed significant anaemia after their admission to ICU (Hb<90g/l) (Table 29).

Overall, the median Hb at ICU admission was 111g/l (IQR 93, 125). Hb was similar between types of admission (Elective Surgery Hb 119g/l, Emergency Surgery 120g/l, Non-operative 114g/l). The median time to significant anaemia (<90g/l) was “Day 2” (Figure 40). There was a wide range of nadir haemoglobin (med 84g/l (IQR 75, 100) (Figure 41). The nadir Hb was <90g/l for 58.1% (n=162) of patients, <80g/l for 39.1% (n=109), and <70g/l for 31 patients (11.1%). The median Hb prior to transfusion was 81g/l (IQR 74, 91) (Figure 44). When patients who received more than two units of RBC at an event were excluded (a surrogate for patients who may have been actively bleeding), the median Hb pre-transfusion was 79g/l (74, 88) (Figure 45). 181 patients were not transfused during the first ten days after their ICU admission and had a wide range of nadir Hb (Figure 43). 79 (48.8%) patients with Hb<90 were not transfused, 39 (35.8%) patients Hb<80, and 4 patients <70 (12.9%).

98 (35.1%) patients admitted to ICU received a RBC transfusion. For these 98 patients, there were 189 transfusion events, and a total of 495 units were transfused. 48 patients were transfused on more than one occasion (med 1; Q1, Q3, max: 1,2,7). The majority of patients received 1-2 units at each transfusion event

(Figure 46). Patients who were transfused received a median of 3.5 units during the first ten days after admission to ICU (Table 30).

Table 29: Baseline characteristics stratified by nadir haemoglobin after ICU admission. "High" >90g/l, "Int" 70-90g/l, "Low"<70g/l. Chi2 test for categorical variables, t-test for parametric continuous variables, Kruskal-Wallis for non-parametric continuous variables. CVD groups: patients can be in more than one subgroup.

	High	**%**	**Int**	**%**	**Low**	**%**	**p test**
N	115	(41.2)	131	(47.0)	31	(11.1)	
Age mean(SD)	72.5	(10.5)	73.4	(11.1)	70.4	(11.4)	0.37
Male sex n(%)	88	(76.5)	89	(67.9)	23	(74.2)	0.314
CVD n(%)							
ACS	8	(7.0)	9	(6.9)	5	(16.1)	
Chronic cardiac disease	73	(63.5)	79	(60.3)	17	(54.8)	
CVA/TIA	19	(16.5)	21	(16.0)	2	(6.5)	
PVD	18	(15.7)	28	(21.4)	9	(29.0)	
>75, DM/BP	36	(31.3)	48	(36.6)	4	(12.9)	
System diagnosis n(%)							0.039
Respiratory	32	(27.8)	30	(22.9)	2	(6.5)	
CVS	19	(16.5)	34	(26.0)	12	(38.7)	
GI	38	(33.0)	38	(29.0)	8	(25.8)	
Neuro	5	(4.3)	6	(4.6)	1	(3.2)	
Genito-urinary	12	(10.4)	14	(10.7)	5	(16.1)	
other	9	(7.8)	9	(6.9)	3	(9.7)	
Comorbidity							
Apache: 0	96	(83.5)	112	(85.5)	23	(74.2)	0.370
1	16	(13.9)	13	(9.9)	7	(22.6)	
≥2	3	(2.6)	6	(4.6)	1	(3.2)	
FCI med(IQR)	2	(1, 3)	2	(1, 4)	2	(1,3)	0.32
Type of admission n(%)							0.111
Elect Surgical	9	(7.8)	8	(6.1)	7	(22.6)	
Emerg Surgical	34	(29.6)	35	(26.7)	12	(38.7)	
Emerg Medical	64	(55.7)	61	(46.6)	15	(48.4)	
APII score	18	(15, 21)	19	(15, 23)	23	(17, 26)	0.057
Vasopress. n(%)	36	(32.4)	53	(41.1)	14	(45.2)	0.265
Mech Vent n(%)	48	(43.6)	70	(53.3)	15	(48.4)	0.316
RRT n(%)	2	(1.8)	3	(2.3)	2	(6.5)	0.349
Admission Hb g/l med (IQR)	124	(115, 135)	97	(98, 112)	91	(67, 112)	
Nadir Hb	103	(97, 110)	78	(75, 83)	66	(62, 68)	
Admission TnI ng/l med (IQR)	35	(10, 137)	37	(14, 127)	32	(9, 239)	0.97
Max Troponin ng/l med (IQR)	66	(17, 212)	126	(31, 403)	164	(52, 924)	0.016
ECG ischaemia n(%)	27	(23.5)	40	(30.5)	12	(38.7)	0.195

Table 30: Pattern of anaemia during first 10 days after ICU admission (includes patients after discharge to the ward)

	Median	Q0, Q1, Q3, Q4
Hb at admission g/l	111	(54, 93, 125, 175)
Hb by admission type g/l		
Elect Surgery	107	(67, 89, 125, 154)
Emerg Surgery	117	(62, 105, 131, 171)
Emerg Medical	111	(54, 95, 125, 175)
Days to Hb<90	2	(0, 1, 4, 10)
Nadir Hb g/l	84	(51, 75, 100, 138)
Patients transfused n(%)	98	(35.1)
Pre-transfusion Hb	79	(54, 74, 89, 159)
Days to first transfusion	3.5	(1, 2, 7, 9)
No RBC units per patient	3.5	(1, 2, 6, 32)

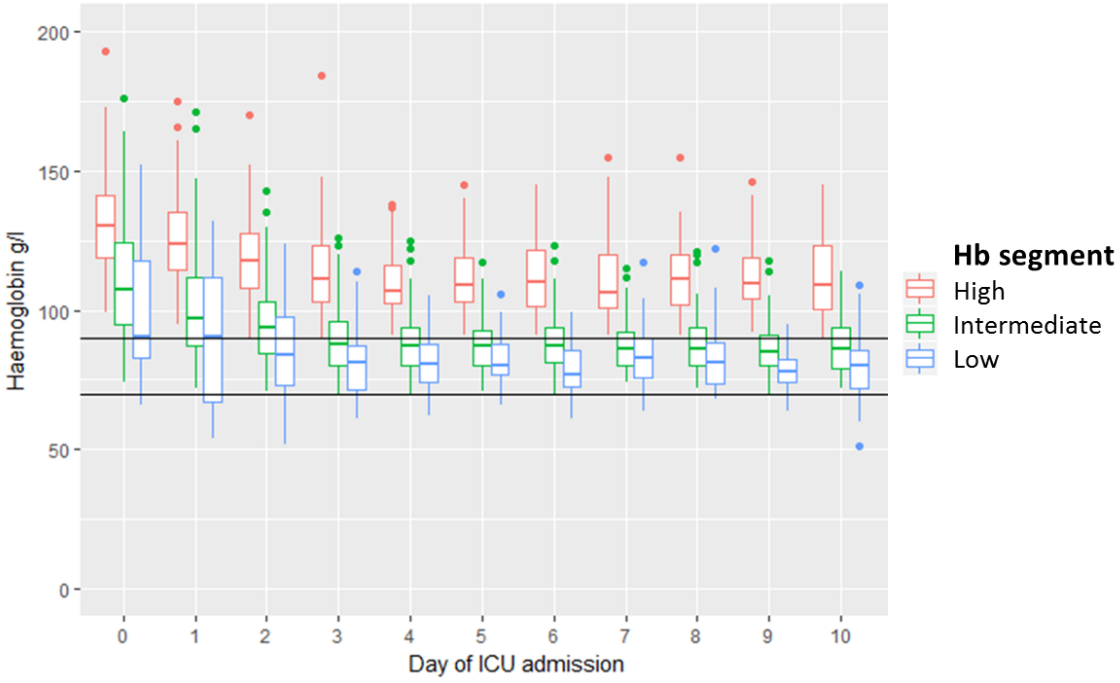

Figure 40: Haemoglobin by day of ICU admission. Horizontal lines at traditional transfusion thresholds Hb=90g/l and Hb=70g/l. Stratified by nadir Hb segment for each patient: red high>90g/l, green intermediate 70-90g/l, blue low<70g/l. Includes patients after discharge to hospital ward. Horizontal lines at 70g/l and 90g/l representing standard transfusion thresholds.

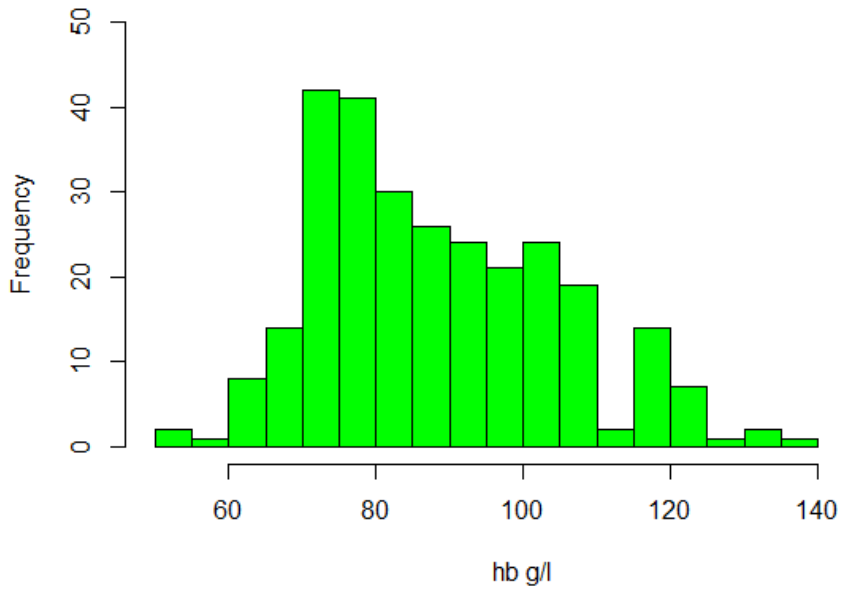

Figure 41: Lowest Hb (g/l) for each patient, whole cohort

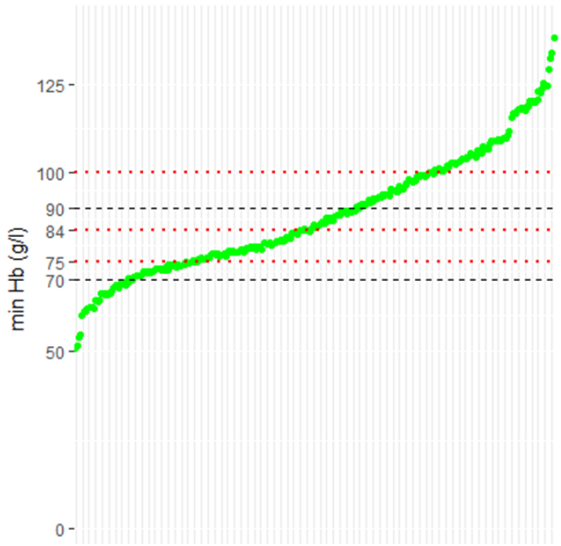

Figure 42 Nadir Hb for whole cohort. Min: 51g/l, Q1 75g/l, Median 84g/l, Q3 100g/l, max 138g/l. 58.1% (n=162) of patients with nadir Hb<90g/l, 39.1% <80g/l, 11.1% <70g/l

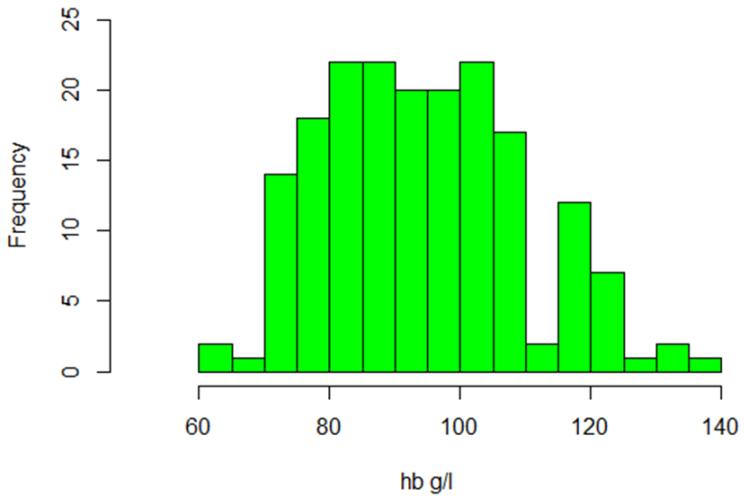

Figure 43: Nadir Hb (g/l) for patients not transfused

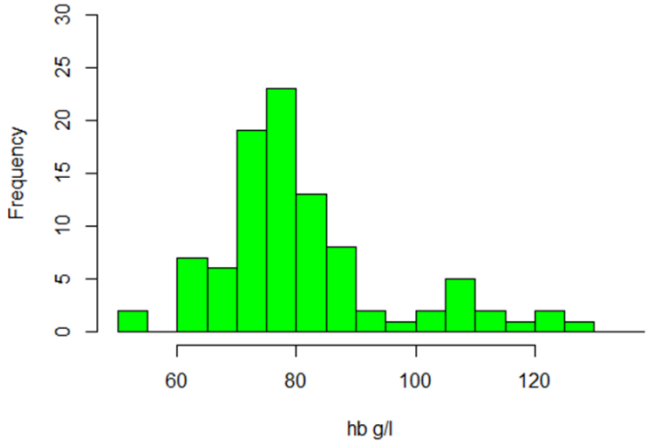

Figure 44: Transfusion trigger. Pre-transfusion Hb (g/l). All transfused patients

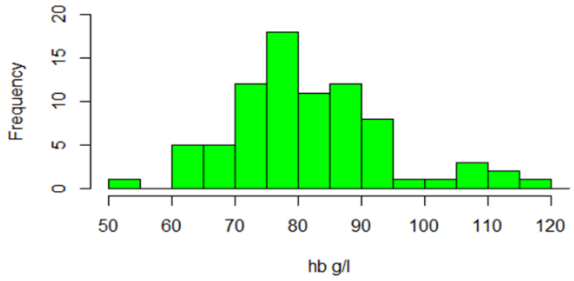

Figure 45: Transfusion trigger. Pre-transfusion Hb (g/l) for patients presumed not actively bleeding [Patients received 1-2 units RBCs] (transfusion events n=144. Transfusion events >2 units: n=45)

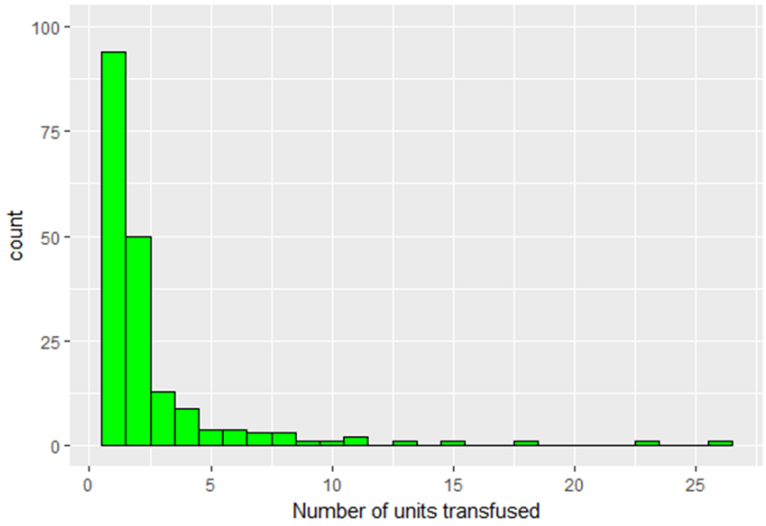

Figure 46: Number of units of RBCs transfused at each event.

5.4.9.1. Association between exposure to anaemia and peak TnI

162 patients were exposed to significant anaemia (Hb<90g/l). Of these 162 patients, 80 patients (49.4%) had a ≥20% rise in TnI after their Hb fell below 90g/l (Figure 48). A further 13 patients had an elevated TnI where there was no preceding TnI, or the preceding TnI was lower, but already elevated above the diagnostic threshold.

5.4.9.2. Association between peak TnI and nadir Hb (whole cohort)

There was a weak univariable association between peak TnI and nadir Hb (whole admission preceding peak TnI), p=0.033 (Figure 47), with a lower Hb associated with a higher peak TnI. Anaemia preceded TnI in 80 patients (49.4%) (Figure 48). When restricted to Hb values in the 24 hours preceding the peak TnI, Hb was considerably higher than the nadir Hb for each patient (Figure 49). However, there was no difference between categories of myocardial injury (p=0.463Figure 46, Figure 50). Patients with Infarction were more likely to have a low Hb in the preceding 24 hours, and this was particularly noticeable for the group whose lactate in this time period was >4.0mmol/l (Figure 51).

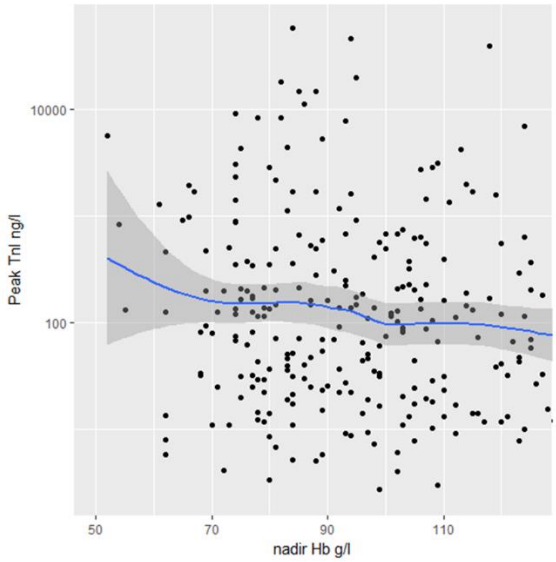

Figure 47: All patients: Exposure to anaemia prior to peak TnI. Scatterplot for nadir Hb (g/l) preceding peak TnI (ng/l, logarithmic scale). p=0.033. Spearman's correlation coefficient r=-0.12

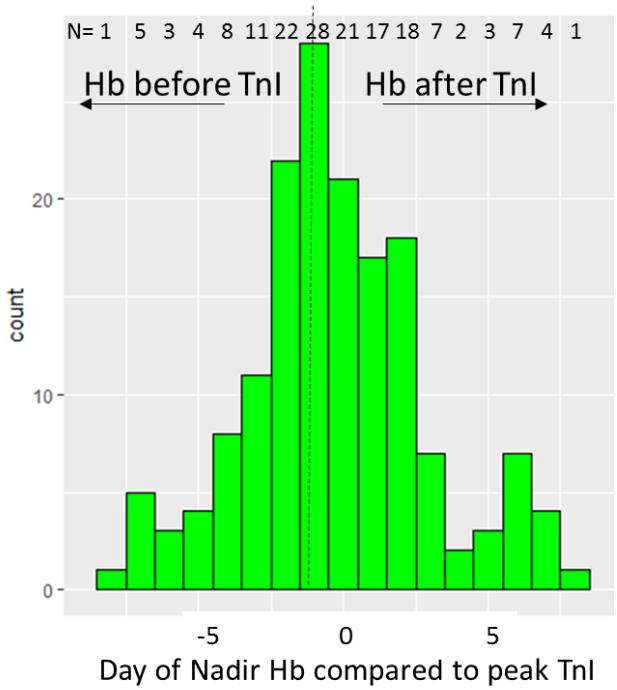

Figure 48: Time difference between peak TnI and exposure to significant anaemia (Hb<90g/l). Anaemia preceded TnI in 80 patients (49.4%). Total patients with nadir Hb<90g/l n=162

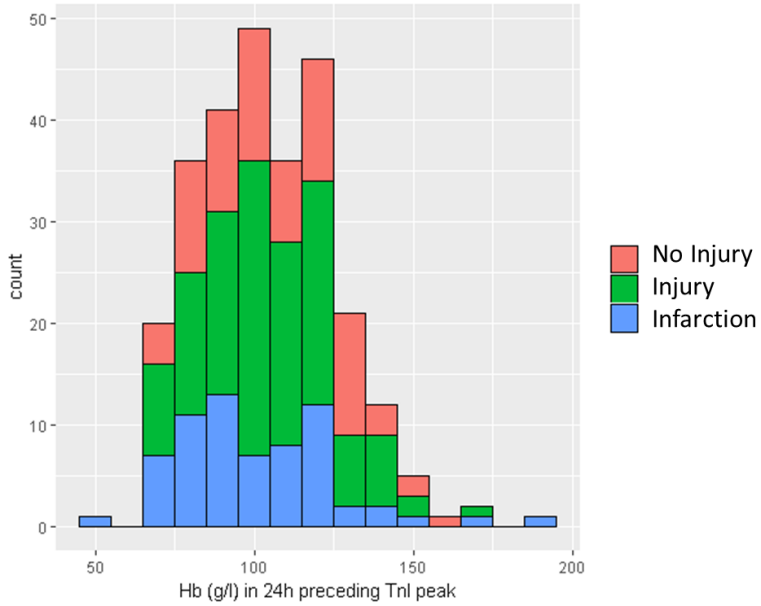

Figure 49: Lowest Hb (g/l) in 24h preceding TnI peak. Categorised by myocardial injury category

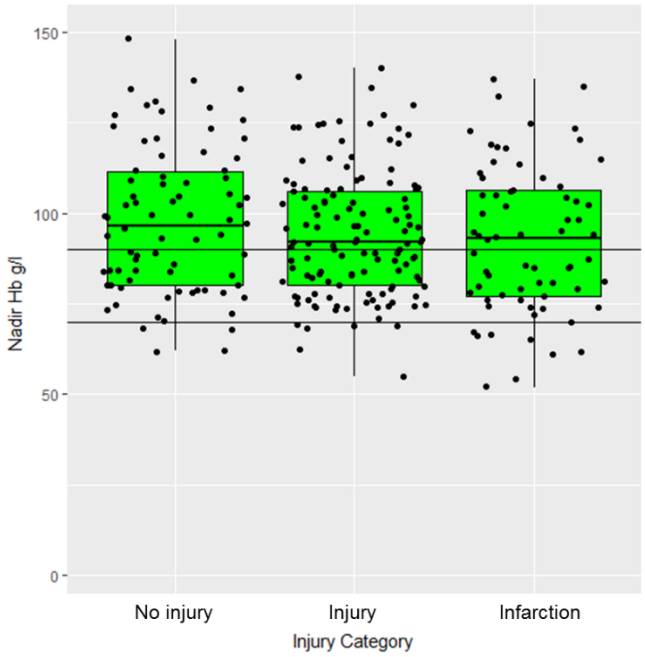

Figure 50: Whole cohort. Box plot for myocardial injury and exposure to anaemia preceding peak TnI. Myocardial injury category vs nadir Hb (g/l). Horizontal lines at 70g/l and 90g/l. Kruskal-Wallis p=0.463

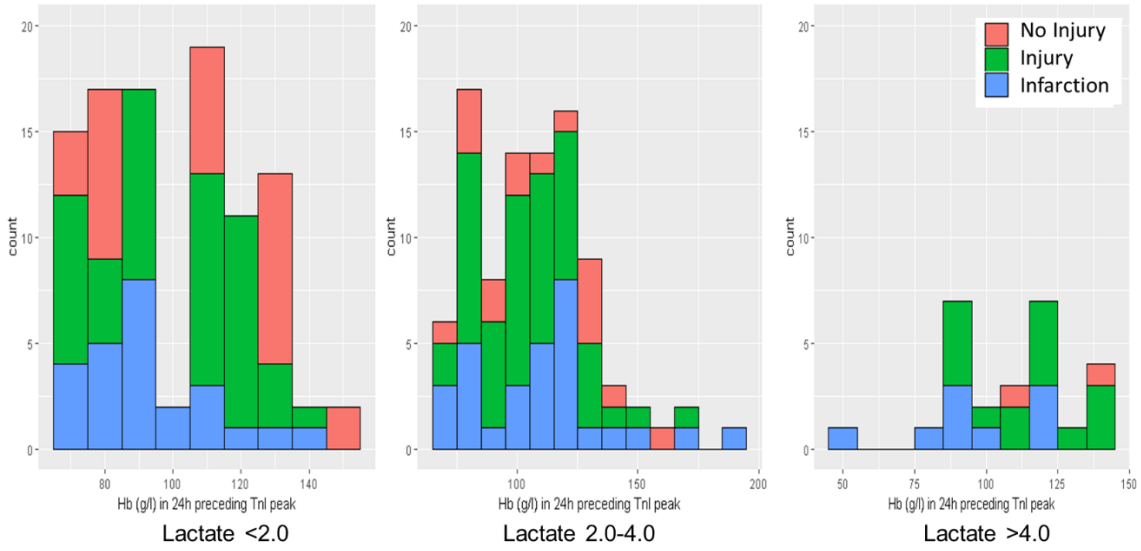

Figure 51: Histogram of distribution of Hb (g/l) in 24 hours preceding peak TnI. Stratified by lactate in same time period: "Low" <2.0mmol/l, "Middle" 2.0-4.0mmol/l, "High" >4.0mmol/l. Coloured by myocardial injury category.

5.4.9.3. Summary

Anaemia was extremely common in this cohort, and 58.8% of patients experienced significant anaemia with a nadir Hb<90g/l. Red blood cell transfusion in the first ten days after ICU admission was common (35.1%). There was a wide range of transfusion practice, with a median pre-transfusion Hb of 79g/l. Most patients received one or two units at a time, with a median of 3 units in total during the first ten days. There was a significant weak univariable association between low Hb and peak TnI, which supports the hypothesis that myocardial injury may be secondary to ischaemia in this population. However, there was no difference between the Injury and Infarction categories.

5.4.10. Severity of illness

The median APACHE II score during the first 24 hours of ICU admission was 18 (IQR 15, 23), and the median SOFA score was 5 (3, 8). Severity of illness peaked between day 2 (med 7, IQR 4, 10) and 3 (med 7, IQR 4, 10) (Figure 52).

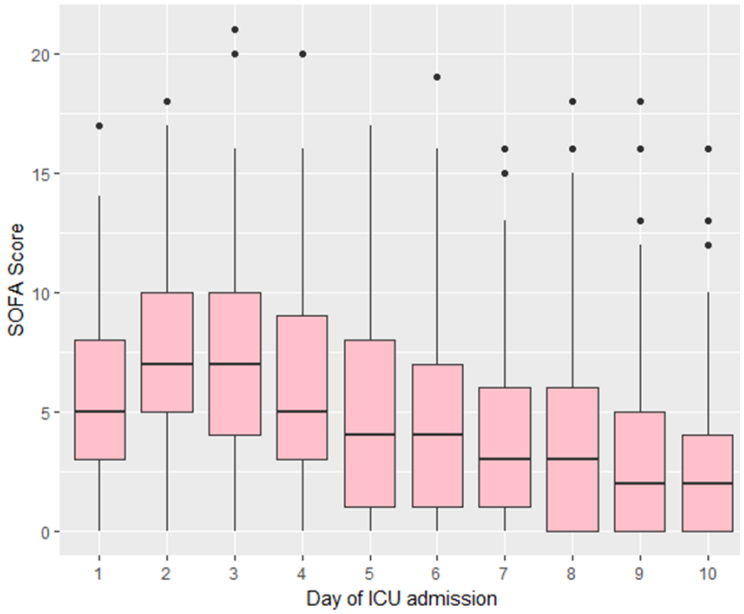

Figure 52: Box plot for Severity of Illness (SOFA Score) by day after ICU admission (n=279). Day 1 = worst scores in first 24 hours in ICU. SOFA score still calculated on ward, with physiological components assumed to be normal. 21 (7.5%) patients died within 10 days after admission to ICU.

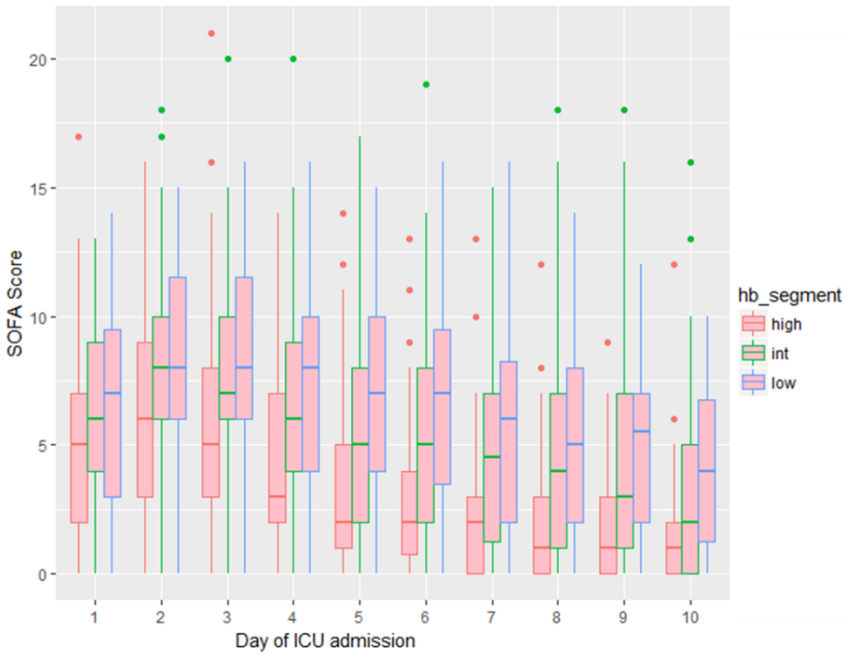

Figure 53: Box plot for SOFA Score vs Day of ICU admission. Stratified by nadir Haemoglobin segment: Red: high >90g/l, Green: intermediate 70-90g/l, Blue: low <70g/l. Kruskal-Wallis p<0.001. 21 (7.5%) patients died within 10 days after admission to ICU

There was a significant difference in severity of illness between patients with different nadir Hb concentrations ($p<0.001$). Hb is not a component of SOFA, so there is no mathematical linkage between these variables. Patients whose Hb concentration remained >90 had a median peak SOFA score of 7 (IQR 4, 10), the intermediate group (70-90g/l) had a peak SOFA of 9 (7, 12), and the low group (<70g/l) had peak SOFA of 9.5 (7, 12).

5.4.10.1. SOFA components and TnI

There was a significant univariable association between peak TnI and SOFA score in the 24 hours preceding the peak ($p<0.001$, Figure 54). When assessing the correlation of TnI with the constituent parts of SOFA, the renal component was most strongly correlated ($r=0.37$), followed by cardiovascular ($r=0.29$), and respiratory ($r=0.23$). Bilirubin and coagulation were weakly correlated, and the CNS component had no correlation with peak TnI (Table 31).

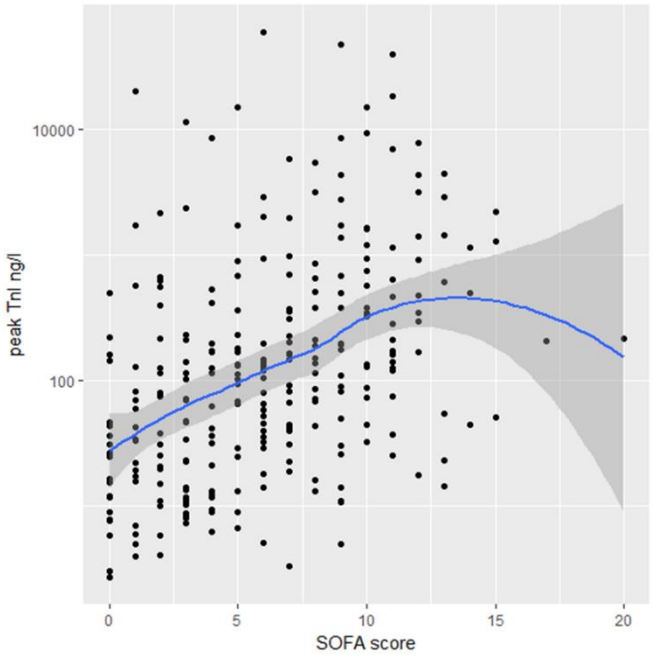

Figure 54: Peak TnI (ng/l) and Severity of Illness Score (SOFA Score) in the 24 hours preceding peak TnI

Table 31: The association of Peak TnI with components of the SOFA Score. Peak TnI (median, min, Q1, Q3, max) for level of category in SOFA score. Correlation between peak log2(TnI) and components of the SOFA Score. R: Pearson's correlation coefficient.

SOFA component	Peak TnI (median)	Min, Q1, Q3, max	R	p value
Respiratory			0.23	<0.001
0	67	(3, 14, 214, 58820)		
1	172	(3, 58, 921, 9292)		
2	93	(3, 26, 218, 47080		
3	126	(12, 31, 433, 7776)		
4	176	(4, 65, 566, 6968)		
CVS			0.29	0.009
0	104	(5, 25, 305, 39600)		
1	66	(4, 22, 321, 8376)		
2	-	-		
3	157	(3, 47, 258, 58820)		
4	130	(3, 30, 888, 47080)		
Renal			0.37	<0.001
0	33	(3, 14, 178, 6968)		
1	149	(8, 65, 643, 58820)		
2	168	(3, 58, 1710, 47080)		
3	160	(34, 106, 262, 899)		
4	216	(9, 68, 1132, 14840)		
Bilirubin			0.13	0.016
0	81	(3, 22, 271, 19860)		
1	88	(4, 23, 214, 58820)		
2	276	(6, 63, 1458, 39600)		
3	134	(9, 50, 509, 843)		
4	14	-		
CNS			0.00	0.759
0	105	(3, 25, 357, 58820)		
1	203	(5, 35, 565, 14980)		
2	197	(4, 82, 1442, 14840)		
3	46	(21, 33, 108, 1132)		
4	38	(14, 14, 86, 160)		
Coagulation			0.18	0.081
0	74	(3, 23, 225, 58820)		
1	92	(3, 20, 469, 39600)		
2	468	(5, 169, 1442, 18380)		
3	51	(13, 32, 66, 125)		
4	140	(68, 104, 177, 213)		

5.4.11. Objective 5: To determine the independent variables associated with TnI elevation.

Hypothesis: Variables that represent ischaemia are independently associated with peak TnI.

We included variables that had a significant univariable association with peak TnI and/or were clinically important to keep in the model. Our variables representing ischaemia were lactate, Haemoglobin, and ECG ischaemia. We restricted the dataset to the 24 hours preceding peak TnI as the time from insult to TnI peak is approximately 12 hours. CRP was not included in this model as it was only performed on a subset of the whole dataset, and did not have a significant univariable association with peak TnI.

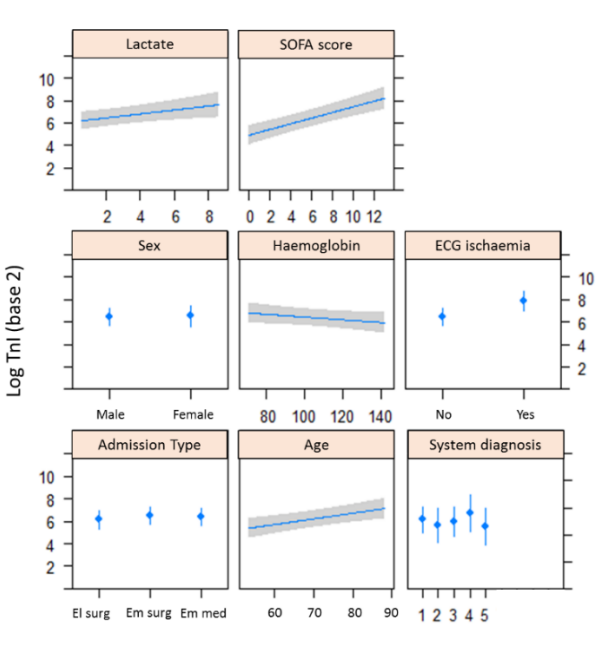

Figure 55: Predictors of Peak TnI in preceding 24 hours. TnI entered as base 2 log(TnI).

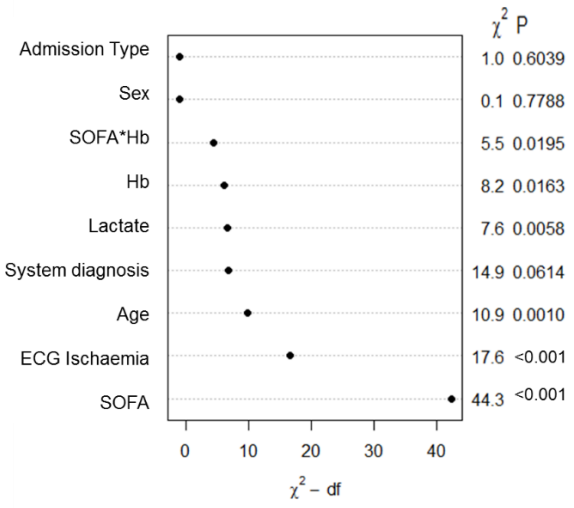

Figure 56: Contribution of predictor variables to overall Chi² for overall model. The larger the Chi2, the greater the contribution to the model. The p value is taken from the Chi2 distribution, and is the probability of seeing a result as extreme in a collection of random data.

SOFA in the preceding 24 hours was the strongest predictor of peak TnI (Chi2 44.3, p<0.001) (Figure 55, Figure 56,
Table 32). For every increase in one point of SOFA, there was an increase in log2(TnI) of -0.10. This is equivalent to a 0.93% increase in the geometric mean of TnI. Dynamic ischaemia on the ECG was also strongly predictive of peak TnI (Chi2 17.6, p<0.001). The presence of dynamic ischaemia was associated with an increase in log2(TnI) of 1.49, or a 2.81% increase in the geometric mean of TnI. Age, haemoglobin and lactate were also significant predictors of peak TnI. There was a significant association for the interaction between SOFA and Hb (p=0.020).

Table 32: Predictors of Peak TnI in preceding 24 hours, limited to first 5 days of ICU admission. Coefficients are pooled estimates using multiple imputation. Coefficient and 95% confidence intervals represent the absolute increase in log2(TnI) for each unit increase in variable. The "t-statistic" is the coefficient divided by its standard error. The p value is taken from the t-distribution, and is the probability of seeing a result as extreme in a collection of random data. AIC 1283. R^2(adj) 0.297

	Coefficient	**Lower 95% CI**	**Upper 95% CI**	**t statistic**	**P value**
Intercept	3.73	0.09	7.36	2.01	
SOFA	0.25	-0.17	0.34	6.10	<0.001
Hb	-0.03	-0.05	0.00	-2.14	0.046
Ischaemia on ECG	1.49	0.81	2.17	4.31	<0.001
Admission type (ref: Elective Surgery)					
Emerg Surgery	0.36	-0.57	1.28	0.75	0.452
Emerg Medical	0.37	-0.49	1.23	0.85	0.398
Age (10yrs)	0.48	0.19	0.77	3.28	0.002
Lactate	0.17	0.04	0.31	2.5	0.005
Female	0.15	-0.53	0.83	0.44	0.842
Diagnosis (ref: Respiratory)					
Cardiovascular	-0.27	-1.28	0.75	-0.52	0.6043
GI	-0.15	-1.05	0.76	-0.32	0.7501
Renal	0.32	-0.79	1.42	0.56	0.5731
Other	-0.42	-1.52	0.69	-0.73	0.4634
SOFA*Hb					0.020

5.4.11.1. Summary

SOFA was most strongly associated with peak TnI. We found that our variables that represented ischaemia were also independently associated with peak TnI. This supports the hypothesis that TnI release in this population may be at least in part a result of ischaemic supply-demand imbalance. This also offers potential variables to target in a randomised controlled trial with the aim of reducing TnI release. Hb concentration and physiological parameters included within SOFA (such as MAP, and heart rate) are potentially modifiable, and manipulation of these may reduce TnI release. The significant association between SOFA and Hb suggests that anaemia (the surrogate for oxygen delivery) was particularly important when the patient was more unwell, potentially when oxygen consumption was highest. This supports the hypothesis that oxygen supply demand imbalance, or type II myocardial infarction is important in critically ill patients with CVD.

5.4.12. Objective 6: To explore whether myocardial injury has an independent association with the outcomes of critically ill patients with CVD.

Hypothesis: Patients with myocardial injury have higher mortality up to six months, and longer ICU and hospital stays after adjusting for important patient factors including severity of illness.

5.4.12.1. Outcomes

37 (13.3%) patients died during their ICU admission, 59 (21.1%) died during their hospital admission, and 78 (28.0%) patients were dead at six months (Table 33, Figure 57). The median length of stay was 6 days in ICU, and (from ICU admission) 18 days in hospital. Patients were ventilated for a median of 2 days. Patients who died in ICU were more likely to have met the "age>75 with DM/BP" inclusion criterion, be emergency non-operative admissions and have higher presenting severity of illness scores. Peak TnI was significantly higher for non-survivors than for the cohort overall (Table 34). There was a competing risk of death for two patients who died on the day of peak TnI and were classified as myocardial injury (Figure 16).

When stratified by myocardial injury category, six month mortality was highest for the Infarction group (n=24, 36.4%), lower for the Injury group (n=39, 31.0%), and lowest for the No Injury group (n=14, 16.7%) (Table 25). Patients with Injury and Infarction had longer duration of mechanical ventilation, and length of ICU and hospital stay.

Table 33: Outcomes for whole cohort. LOS Length of Stay, MV Mechanical Ventilation

	n/median	% or Q0,Q1,Q3,Q4
ICU mortality	37	(13.3%)
Hospital mortality	59	(21.1%)
6 month mortality	78	(28.0%)
ICU LOS (days)	6	(2, 4, 13, 31)
Duration MV (days)	2	(0, 0, 6, 39)
Hospital LOS (days)	18	(1, 11, 34, 157)

Table 34: Admission characteristics stratified by mortality at ICU, hospital and six months

	ICU mortality	%	Hospital mortality	%	6 month mortality	%
total	37	(13.3)	59	(21.1)	78	(28.0)
Age mean(SD)	74.2	(8.2)	75.3	(8.3)	73.8	(9.8)
Male sex n(%)	22	(11.0)	36	(18.0)	50	(25.0)
Female sex n(%)	15	(19.0)	23	(29.1)	28	(35.4)
CVD						
ACS	3	(13.6)	5	(22.7)	8	(36.4)
Chronic	16	(9.2)	31	(17.8)	49	(28.2)
CVA/TIA	5	(11.9)	8	(19.0)	13	(31.0)
PVD	8	(14.5)	12	(21.8)	17	(30.9)
>75	20	(22.2)	24	(26.7)	31	(34.4)
Admission type						
El Surg	4	(7.1)	7	(12.5)	13	(23.2)
Em Surg	7	(8.6)	14	(17.3)	16	(19.8)
Non-Op	26	(18.3)	38	(26.8)	49	(34.5)
Apache II	24	(19, 28)	22	(18, 27)	21	(18, 26)
Peak TnI (ng/l)	169	(66, 554)	176	(54, 899)	169	(44, 600)

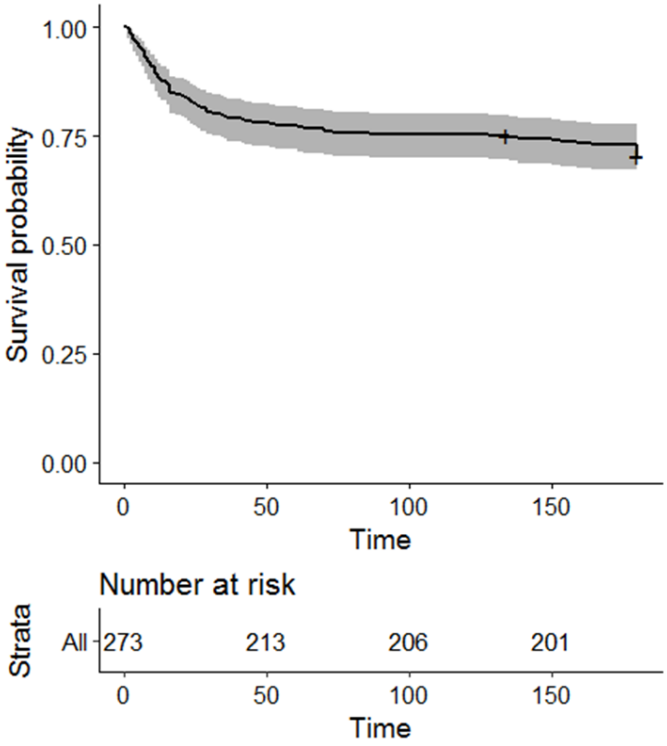

Figure 57: Kaplan-Meier plot with 95% CI of survival for the TROPICCAL cohort. All patients had complete six month follow up. There were 82 deaths over the six month period.

5.4.12.2. Univariable analysis

Univariable associations with six month survival using the log rank test were not significant for ICU admission diagnosis (p=0.211), type of cardiovascular disease (p=0.511), or sex (p=0.051) (see appendix). APACHE II score was most strongly associated with six month survival (Figure 58, p=0.003), followed by TnI (Figure 59) p=0.003), lactate (Figure 60, p=0.015) and Hb segment (Figure 61, p=0.016). Patients with injury and infarction had higher mortality rates up to six months than patients with no injury, Figure 62). However, there was no significant difference between the injury and infarction categories (Injury: no dynamic ischaemic ECG changes, Infarction: dynamic ECG changes consistent with ischaemia, p=0.292).Stratifying the analysis by whether the patient was "more severely ill (APACHE II Score > 18 (median for cohort)), or "less severely ill" (APACHE II Score <=18) showed that the impact of infarction seemed to have a greater independent influence in the less severely ill group (Figure 63).

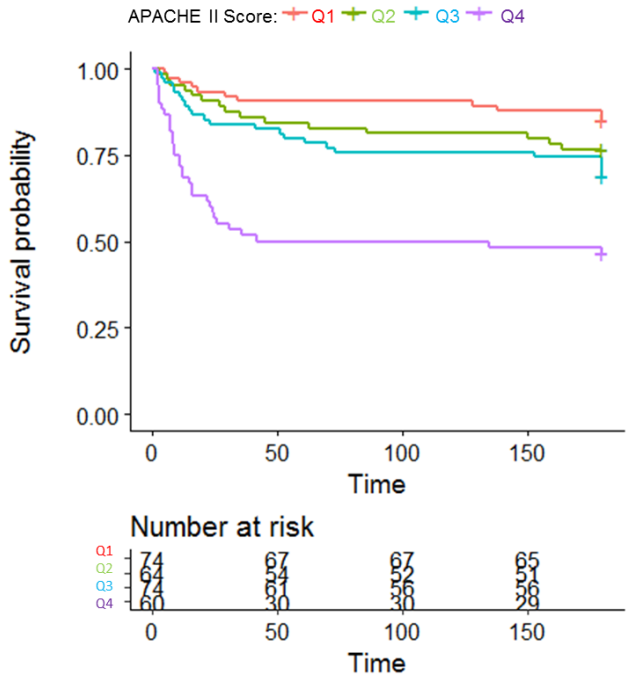

Figure 58: KM for Severity of illness (apache II quartiles Q1 <=15, Q2 16-18, Q3 19-23, Q4>23). Log rank test Chi2=32.1 (df=3) p=0.003

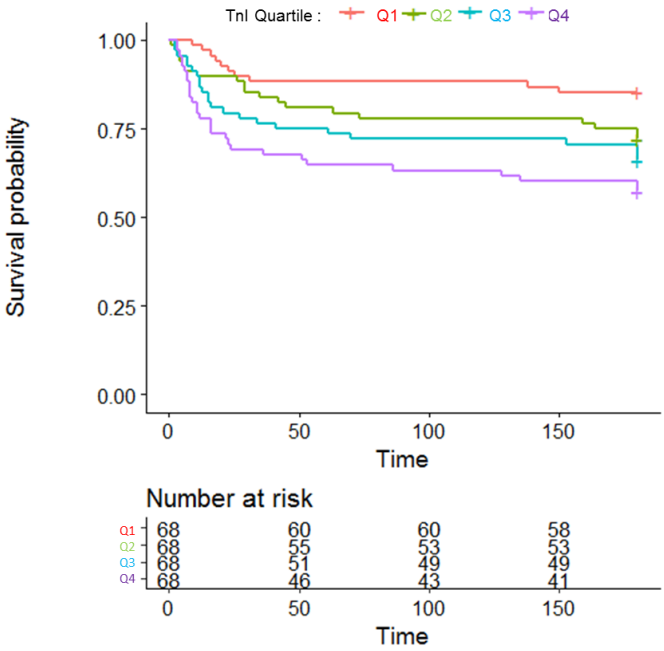

Figure 59: KM plot for TnI quartiles: "Q1"<25ng/l, "Q2" 25-107ng/l, "Q3" 107-380 ng/l, "Q4" >380ng/l. Log rank test Chi2=14.1 (df=3); p=0.003

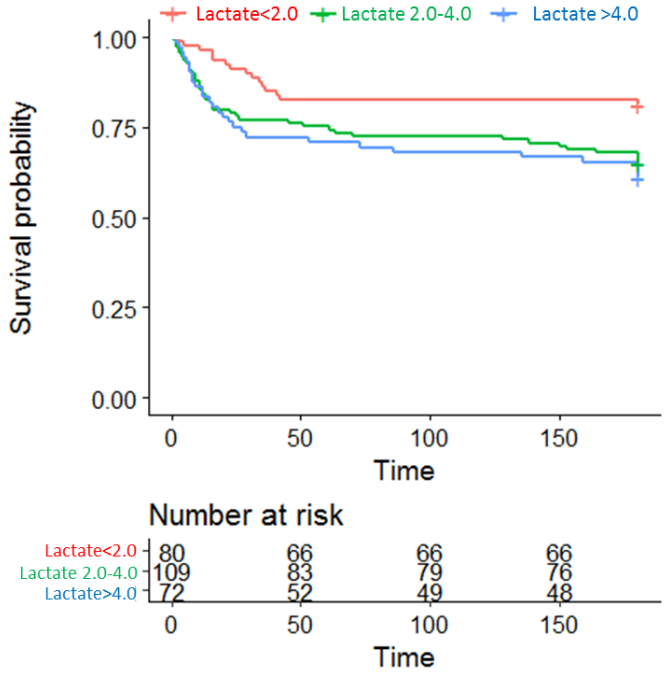

Figure 60: KM plot stratified by Lactate category: "0" <2mmol/l, "1" 2-4mmol/l, "2" >4mmol/l. Log rank test Chi2=8.4 (df=2), p=0.015

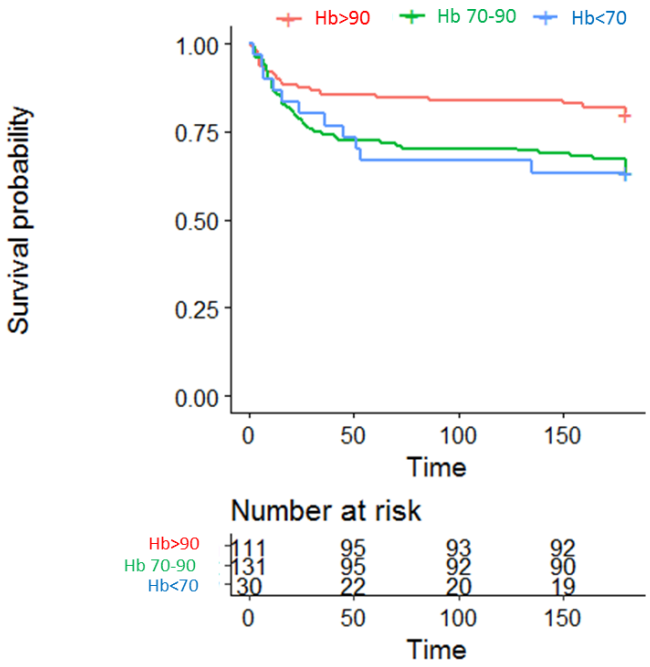

Figure 61: KM plot stratified by Hb Segment: "High" nadir Hb >90g/l, "Int" nadir Hb 70-90g/l, "Low" Hb <70g/l. Log rank test Chi2=8.2, p=0.016

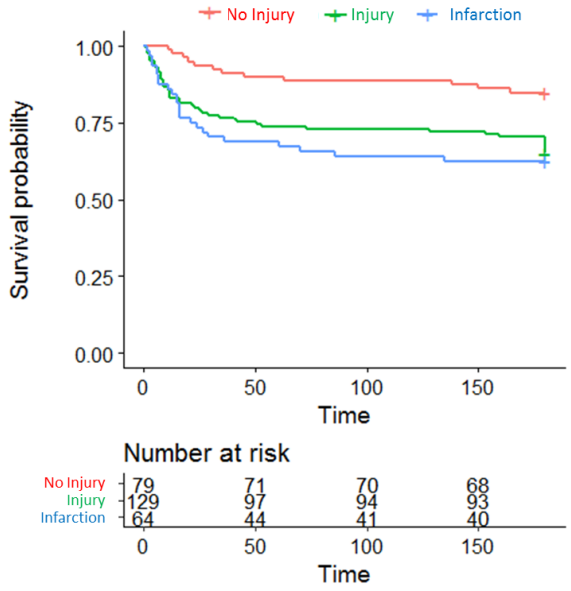

Figure 62: KM plot for Myocardial Injury categories. No Injury, Injury, Infarction. Log rank test Chi2=11.8 (df=2), p=0.003. Log rank test between Injury and Infarction (ie the absence or presence of dynamic changes on the ECG) Chi2=1.1 (df=1), p=0.292.

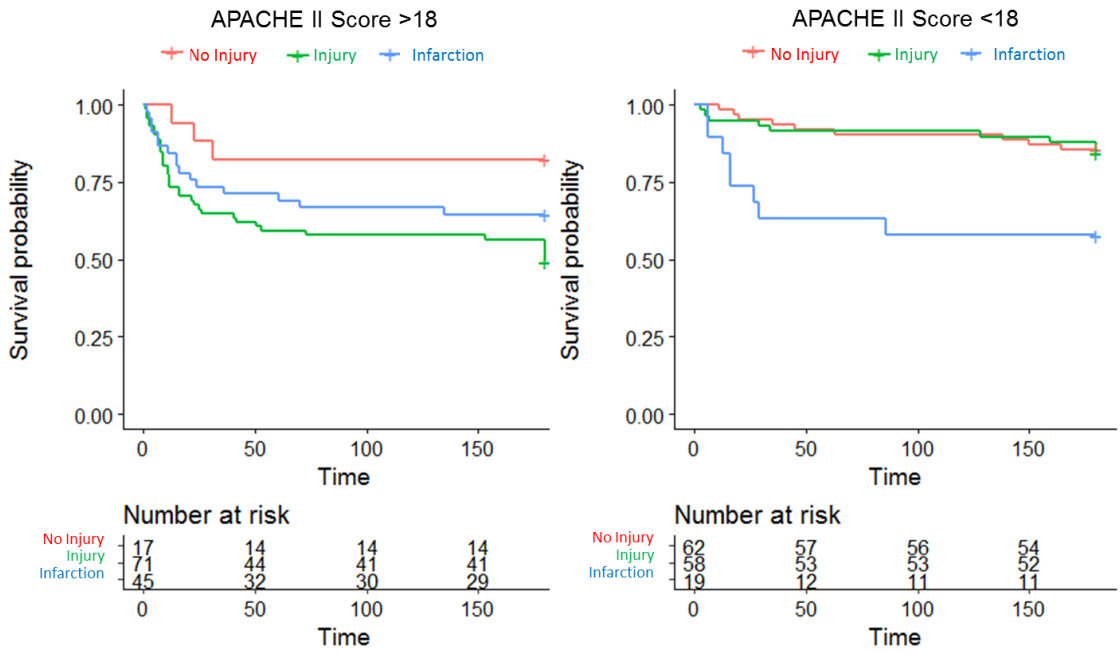

Figure 63: Left: myocardial injury in "more severely ill" patients (APACHE II Score >18 (median)). Log rank test Chi2=6.4 (df=2), p=0.042. Right: myocardial injury in "less severely ill" patients (APACHE II Score <=18). Log rank test Chi2=10.5 (df=2) p=0.005

5.4.13. Multivariable logistic regression 6 month mortality

We included variables which had a significant univariable association with mortality (APACHE score, lactate, nadir Hb), and variables which were clinically important (Sex, Diagnosis). For the primary analysis myocardial injury was entered as a three level categorical variable "No Injury", "Injury", "Infarction". We performed a sensitivity analysis for TnI as a continuous variable with an interaction term for ECG dynamic ischaemia (5.4.13.2). We also performed a sensitivity analysis with higher TnI thresholds for the diagnosis of Injury and Infarction (5.4.13.3). Finally, we performed a sensitivity analysis where we excluded T wave inversion from the diagnosis of dynamic ECG ischaemia as we found this had poor agreement between cardiologists, and was often associated with low peak TnI (5.4.13.4).

5.4.13.1. Primary analysis

The OR for Injury compared with No Injury was 1.55 (95% CI 0.70 to 3.46, p=0.121), and for Infarction compared with No Injury was 2.46 (95% CI 0.98 to 6.15, p=0.20)

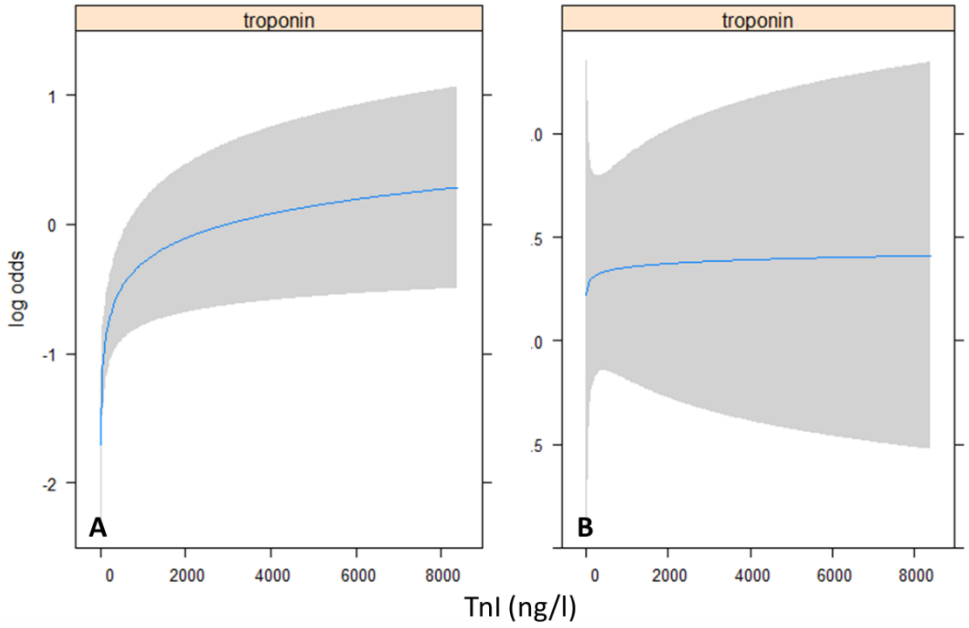

Figure 66: Logistic regression 6 month mortality stratified by ischaemia on ECG. A: plot of TnI vs log odds for no dynamic ischaemic changes OR 1.21, 95% CI 1.08 to 1.36. B: TnI vs log odds for dynamic ischaemia OR 1.02, 95% CI 0.85 to 1.21. TnI entered as TnI^-2 term

Table 36, Figure 64). APACHE Score had a significant independent association with 6 month mortality (OR per 1 point increase 1.05, 95% CI 1.01 to 1.11, p=0.028). Nadir haemoglobin (OR per 10g/l increase 0.62, 95% CI 0.40 to 0.96, p=0.036) also had an independent association with 6 month mortality. The odds of mortality increased with increasing lactate, but the confidence interval crossed 1.0 (OR 1.10, 95% CI 0.98 to 1.22, p=0.078). Sex and diagnosis were not significant predictors of mortality, but were important variables clinically, so were retained.

Table 35: Multivariable Logistic Regression. R^2= 0.196, LR 40.4, c-index 0.736.

	OR	Lower 0.95	Upper 0.95	p value
APACHE score	1.06	1.01	1.11	0.028
Lactate	1.10	0.99	1.22	0.078
Nadir Hb	0.62	0.40	0.96	0.036
Female	1.32	0.72	2.41	0.349
Myocardial Injury (ref: "No Injury")				
Injury	1.55	0.70	3.46	0.121
Infarction	2.46	0.98	6.15	0.202
Diagnosis (ref: "Respiratory")				
CVS	0.69	0.30	1.62	0.294
GI	0.54	0.24	1.21	0.098
Renal	1.05	0.39	2.82	0.985
Other	0.66	0.24	1.82	0.342

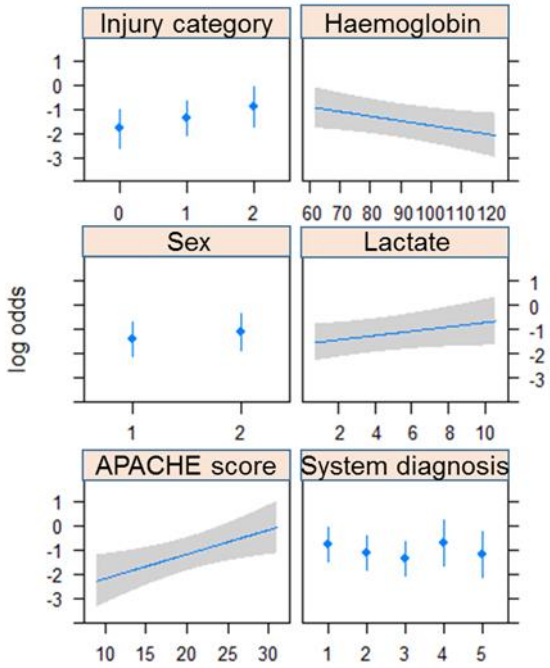

Figure 64: Predictors of mortality at 6 months vs log odds, with 95% confidence intervals for each variable. Injury Category: "0" No Injury, "1" Injury, "2" Infarction. Sex: "1" Male, "2" Female. System diagnosis: "1" Respiratory, "2" Cardiovascular, "3" Gastrointestinal, "4" Renal, "5" Other.

5.4.13.2. Sensitivity analysis: Interaction of TnI with ECG ischaemia

Using myocardial injury as a categorical variable, patients with Infarction had a higher odds ratio for 6 month mortality compared with patients in the Injury category, although the confidence intervals overlapped. We wished to explore whether this could be because interaction with dynamic changes on ECG altered patient outcome, or whether it was because patients with Infarction had higher TnI than patients with Injury.

5.4.13.2.1. Stratification using Kaplan Meier Plots

We entered TnI as a binary variable to assess this interaction because KM plots require categorical variables. The use of quartiles would have resulted in eight small groups, which would have been underpowered. Using univariable KM plots, when we stratified the binary TnI variable by the presence or absence of dynamic ischaemia on the ECG (Figure 65) we found that in the absence of ischaemia, the higher TnI category had a worse survival (p=0.003). However, if ischaemia was present, there was no significant difference between the groups (p=0.498). This potentially suggests that if the patient had an ECG based ischaemic event this was the dominant issue rather than the TnI value (accepting the loss of power with the binary variable), whereas if the patient only had TnI elevation then the magnitude was more closely related to ultimate mortality. This supports the 'construct' of including ECG change as a key part of diagnosis in the population.

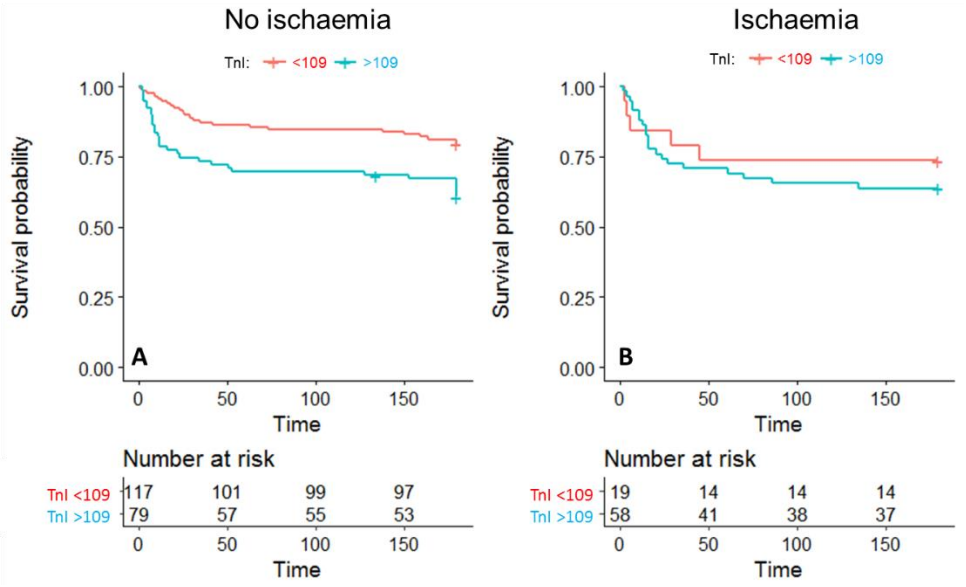

Figure 65: KM plot for Binary TnI (0: <109 (median peak TnI), 1: >109ng/l), stratified by left: no dynamic ischaemia on ECG (log rank Chi2=9.1 (df=1), p=0.0025), right: dynamic ischaemic changes on ECG (log rank Chi2=0.5 (df=1), p=0.498).

This finding was confirmed using logistic regression (Figure 66)). The confidence intervals, particularly for the group with ischaemia were wide, limiting interpretation of these results. In the absence of ischaemia, TnI had a significant association with six month mortality (OR 1.21, 95% CI 1.08 to 1.36), whereas, in the presence of ischaemia, the relationship between TnI and 6 month mortality was no longer significant (OR 1.02, 95% CI 0.85 to 1.21). When added into the same model, the interaction term between TnI and ischaemia was not significant (p=0.109). This may have been because our study was underpowered for this interaction, however it may also be that there was no interaction – that ischaemia had the same association with mortality irrespective of the magnitude of TnI.

5.4.13.2.2. Sensitivity Analysis TnI with ischaemia: Logistic Regression

There was a significant univariable association between TnI and six month mortality (OR 1.30, 95% CI 1.07, 1.58, p=0.010). This was attenuated slightly by the addition of ischaemia (TnI OR 1.26, 95% CI 1.05 to 1.54, p=0.014) and the TnI*ischaemia interaction term (p=0.409) (Table 36). Visual inspection of the log odds plots

for TnI stratified by the presence/absence of ischaemia suggest that the association of TnI and 6 month mortality may be different in patients with Injury and Infarction. However, the confidence intervals are extremely large, reflecting the lack of power in this dataset for this analysis.

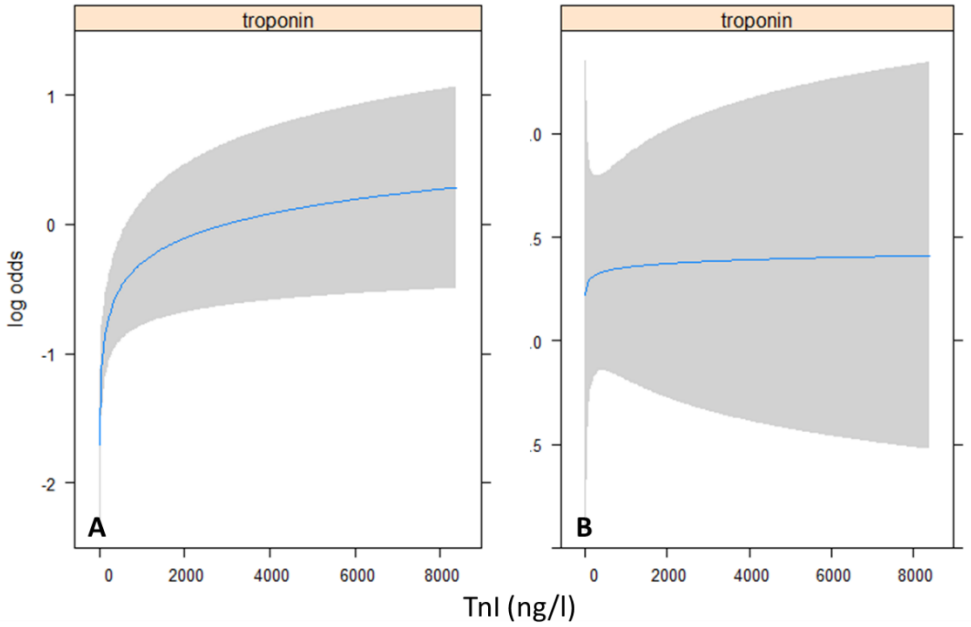

Figure 66: Logistic regression 6 month mortality stratified by ischaemia on ECG. A: plot of TnI vs log odds for no dynamic ischaemic changes OR 1.21, 95% CI 1.08 to 1.36. B: TnI vs log odds for dynamic ischaemia OR 1.02, 95% CI 0.85 to 1.21. TnI entered as TnI^-2 term

Table 36: Multivariable logistic regression for 6 month mortality. R^2 0.20, LR 41.31. TnI entered as TnI$^{\wedge -2}$ as best fit from multiple fractional polynomials. TnI entered as TnI^-2 term, OR represents change in TnI from 1st Quartile to 3rd Quartile.

	OR	Lower 0.95	Upper 0.95	p value
Univariable analysis				
TnI	1.30	1.07	1.58	0.01
Interaction TnI*ischaemia				
TnI	1.27	1.05	1.53	0.014
Ischaemia	1.05	0.58	1.90	0.787
TnI*ischaemia				0.409

1.1.1.1 Summary

Accepting that the analyses were underpowered, the magnitude of TnI had a stronger association with six month mortality for the Injury group (with no dynamic ECG changes), compared to the Infarction group (with dynamic ECG changes consistent with ischaemia). Together with the observations from the Kaplan Meier survival plots, this suggests that if the patient had an ECG based ischaemic event this was the dominant issue rather than the

TnI value, whereas if the patient only had TnI elevation then the magnitude was more closely related to ultimate mortality. This supports the hypothesis that Infarction is a separate category from Injury.

5.4.13.3. Sensitivity analysis: Restrict diagnostic thresholds to x5 Upper Reference Limit for TnI

Restriction of the diagnostic threshold for TnI elevation to five times the upper reference limit resulted in 154 (56.4%) patients being classified as "No Injury", 69 (25.3%) patients as "Injury", and 50 (18.3%) patients as "Infarction". The higher threshold resulted in a higher six month mortality for the No Injury group (21.4% vs 14.3%). Substitution of the new URL in the model for the prediction of six month mortality had minimal impact on the model fit, and the odds ratios for Injury and Infarction became very similar (Table 37).

Table 37: Sensitivity analysis: Increasing the diagnostic threshold for TnI elevation to x5 the standard diagnostic threshold (80ng/l for women, 170ng/l for men). Sensitivity, specificity, positive predictive value and negative predictive value presented as Injury (Injury+Infarction) vs No Injury, and Dead vs Alive at six months.

	Standard threshold	**x5URL**
6 month mortality		
No Injury	12 (14.3%)	33 (21.4%)
Injury	45 (36.3%)	30 (43.5%)
Infarction	24 (36.4%)	18 (36.0%)
Multivariable model statistics		
TnI OR for 6 month mortality		
Injury	1.55 (0.70 to 3.46)	1.69 (0.82 to 3.51)
Infarction	2.46 (0.98 to 6.15)	1.63 (0.69 to 3.84)
R2	0.196	0.191
C-index	0.736	0.728
Brier Index	0.179	0.179

5.4.13.4. Sensitivity analysis: Exclude T wave inversion from definition of Infarction

There were 17 patients who had dynamic T wave inversion and no other ischaemic changes on their ECGs. Exclusion of T wave inversion from the definition of MI resulted in the re-classification of 17 patients from Infarction to Injury, of whom 13 patients survived, and four patients died. Median peak TnI did not change significantly for each group (Injury: median 160ng/l IQR 74, 554; Infarction med 365, IQR 117, 1768). Dynamic ischaemia remained an independent predictor of peak TnI (Figure 67), although the contribution to the overall Chi^2 reduced (from 17.6 to 13.5). Six month mortality was high in the Infarction group (40.4%) compared with Injury (26.5%) and No Injury (15.6%) (Figure 68), and was higher than the original classification (36.4%).

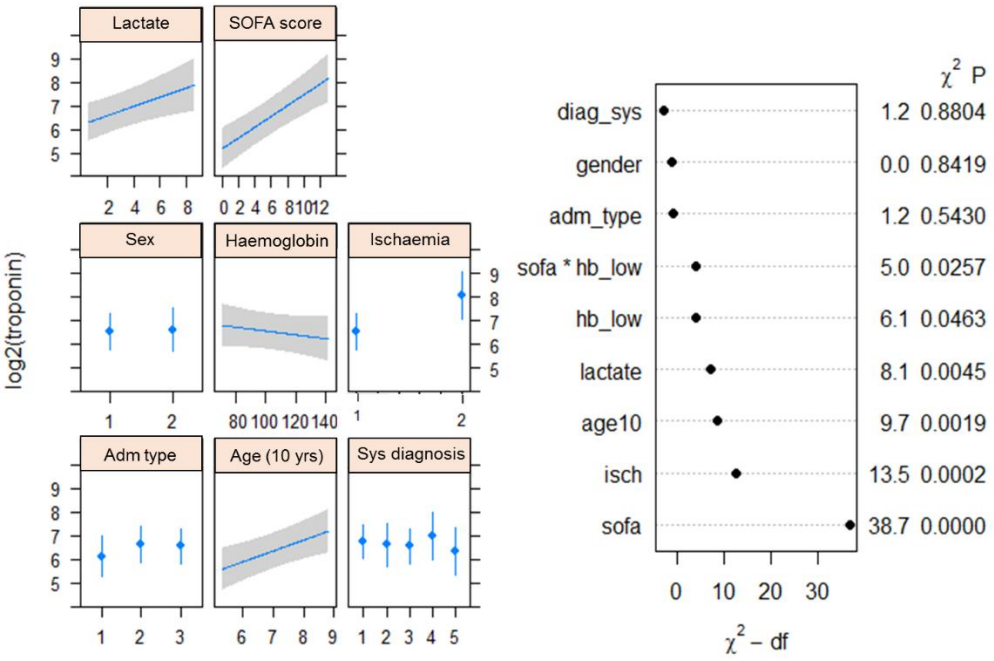

Figure 67: Sensitivity analysis: Predictors of peak TnI. T wave inversion excluded from criteria for Infarction. Sex: "1" Male, "2" Female. Ischaemia: "0" No Ischaemia, "2" Dynamic Ischaemia. Adm type: "1" Elective Surgical, "2" Emergency Surgical, "3" Medical. System diagnosis: "1" Respiratory, "2" Cardiovascular, "3" Gastrointestinal, "4" Renal, "5" Other.

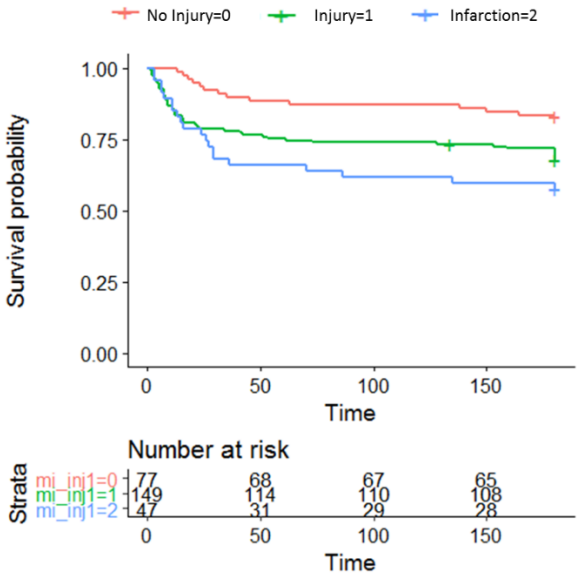

Figure 68: Sensitivity Analysis: KM plot for six month survival stratified by new myocardial injury variable. T wave inversion is excluded from the diagnostic criteria for infarction. Log rank test Chi2 10.7 (df=2), p=0.005

Replacement with the new myocardial injury variable into the model made little difference to the overall fit and discriminatory ability of the model (R^2 0.196 to 0.191, c-index 0.736 to 0.731). The point estimate for the odds ratio for 6 month mortality for the Injury category was reduced from 1.55 (95% CI 0.70 to 3.46, p=0.767) to

1.26 (95% CI 0.58 to 2.73, p=0.229) but the confidence intervals were wide, and overlapped. The OR for Infarction remained the same OR 2.46 (0.98 to 6.15, p=0.039) to 2.47 (0.91 to 6.72, p=0.011).

Table 38: Sensitivity analyses: Odds ratios and 95% CI for logistic regression model with new myocardial injury category, excluding T wave inversion from the diagnostic criteria for Infarction. R2=0.191, c-index=0.731)

Variable	OR	Lower 0.95	Upper 0.95	p value
APACHE Score	1.17	1.03	1.33	0.018
Peak Lactate	1.09	0.98	1.21	0.111
Nadir Hb	0.81	0.68	0.97	0.024
gender - 2:1	1.43	0.77	2.64	0.253
Myocardial Injury (ref: No Injury)				
Injury	1.26	0.58	2.73	0.229
Infarction	2.47	0.91	6.72	0.101
Diagnosis (ref: Respiratory)				
CVS	0.67	0.29	1.53	0.341
GI	0.48	0.21	1.06	0.071
Renal	0.97	0.37	2.59	0.954
Other	0.56	0.20	1.56	0.269

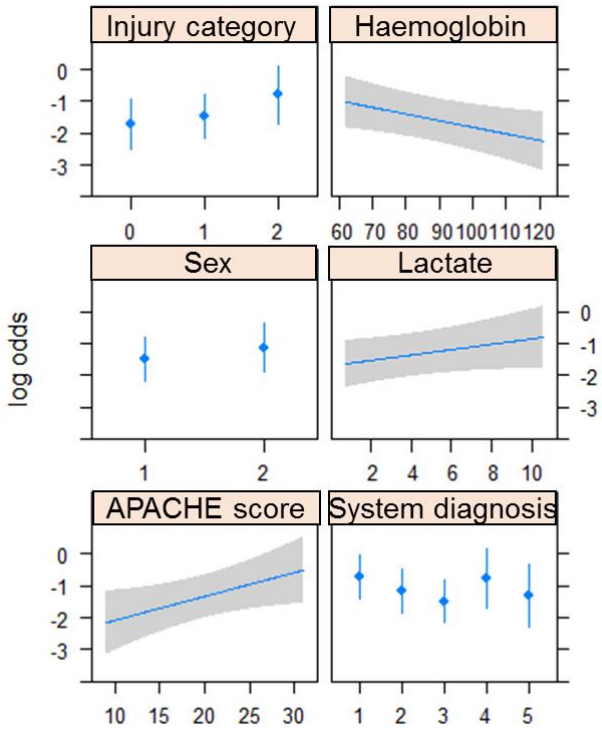

Figure 69: Sensitivity analysis: no T wave inversion: Predictors of mortality at six months. T wave inversion excluded from diagnostic criteria for Infarction. Injury Category: "0" No Injury, "1" Injury, "2" Infarction. Sex: "1" Male, "2" Female. System diagnosis: "1" Respiratory, "2" Cardiovascular, "3" Gastrointestinal, "4" Renal, "5" Other.

5.4.13.5. Summary

We found that TnI as a continuous variable had an independent association with 6 month mortality. Patients with Infarction had higher 6 month mortality than patients with Injury, although this was not significant in the

multivariable model. When we explored the interaction between TnI and ischaemia, we found that ischaemia altered the relationship between TnI and mortality. If there was no ischaemia, then the greater the TnI value, the greater the mortality. However, if ischaemia was present, this appeared to be the dominant event, rather than the magnitude of TnI. Exclusion of T wave inversion from the diagnosis of Infarction led to a clearer distinction between Injury and Infarction for the association with 6 month mortality. This supports the hypothesis that regional ischaemic TnI release (Type I or II MI) is a separate entity in critically ill patients with CVD, which we may be able to target with manipulation of physiological parameters to maximise myocardial oxygen delivery. When we used the higher cut-offs to categorise TnI, we saw greater separation of the groups in terms of survival, which may support the importance of infarction for mortality. The higher cut off may be more relevant pathophysiologically in this population given the multiple mechanisms that contribute to myocardial injury.

5.5. Discussion

Using a high sensitivity assay, we found that TnI was detectable in all critically ill patients with cardiovascular disease who were recruited to TROPICCAL. The rise and fall pattern was consistently seen, which was in keeping with an "acute hit/event" mechanism of injury, rather than an ongoing injury. Using the Third Universal Definition, 27.7% of patients were classified as "No Injury", 45.2% of patients had "Injury", and 24.0% of patients had "Infarction", compared to only 4.4% of patients who were clinically diagnosed with Infarction. The presence of ischaemia on ECG was an independent predictor of peak TnI together with age, SOFA score, haemoglobin and lactate in the previous 24 hours. The lack of association with CRP (a more general marker of systemic inflammation), with stronger association with lactate (a marker of inadequate perfusion/oxygen supply) provide support for the conjecture that injury results from an acute ischaemic hit in this population. Patients with Injury or Infarction had significantly higher ICU and longer-term mortality up to six months than patients with No Injury. Patients with Infarction had similar baseline demographics to patients with Injury, but had higher peak TnI concentrations, and higher hospital and six month mortality. The magnitude of TnI appeared to have a stronger association with six month mortality in the Injury group when there were no dynamic ECG changes. T wave inversion had poor agreement and poor prediction of elevated peak TnI, and there was a clearer distinction between the Injury and Infarction groups when T wave inversion was excluded from the diagnostic criteria for ECG dynamic ischaemia. This supports the importance of including the systematic assessment of new dynamic change on ECG in the myocardial injury 'construct' in ICU. This has implications for subsequent management: further investigation and initiation of ACS treatment should be considered for patients with dynamic TnI elevation above the sex-specific diagnostic threshold and ECG changes consistent with regional myocardial ischaemia, whereas the risk-benefit balance may be different in patients without ECG changes.

5.5.1. Strengths and Limitations

A significant strength of this study was that the cohort included patients from a range of teaching and district general hospitals. We used the same highly sensitive assay across all sites. Patients with chronic cardiac disease accounted for 58.8% of our patients, and we recruited patients from all our CVD categories, enabling us to comment on all the groups. We had complete six month follow up for all our patients. We collected daily TnI results and markers of illness severity, enabling us to look at changes in these markers over time, rather than a fixed arbitrary time point.

We excluded patients with acute intracerebral pathology and patients who presented after cardiac arrest. Acute intracerebral pathology can cause stress cardiomyopathy, which has a distinct pattern and course. After cardiac arrest, TnI can be also elevated as a result of direct myocardial trauma. Exclusion of these patients gave us a cleaner picture of the dynamics of TnI in critically ill patients with CVD, but it means that we are unable to generalise our findings to these groups. We also excluded patients who were receiving palliative care. Inclusion of these patients would have given us a greater insight into the rise and fall of TnI in the very sick and dying patient, but we did not feel it was appropriate to approach these patients or their relatives with research requests.

Approximately 80% of admissions were unplanned emergency admissions, consistent with previous studies (269). Unlike elective surgical patients, where we could have taken TnI pre-op, and timed TnI release in relation to their surgical insult, we were unable to capture these patients at the very beginning of their critical illness.

Admission to ICU is defined by hospital location rather than the stage of illness, and may vary considerably between patients and hospital organisations. As a result, we were unable to use methods such as area under the concentration-time curve, or TnI at specific time points as used in Acute Coronary Syndrome (80) and elective major surgery (270) where the time of onset of symptoms or insult is clearly defined. Not all our elective patients had pre-operative TnI measured, and we have therefore not performed these analyses on this subset.

We measured TnI for ten days, severity of illness for a maximum of ten days, and daily ECGs for the first five days after ICU admission. It is possible that patients had further episodes of myocardial injury or infarction later in their hospital stay that we did not pick up, and that would have altered their outcomes. However, only 12% of patients had a TnI peak in the days after we stopped ECG screening that was greater than TnI in the first six days.

Only 9.3% of patients were on no long-term cardiac medication. Studies looking at beta blockers in patients with known cardiac disease in the peri-operative period suggest that it is beneficial to continue these drugs, and that withdrawal of beta blockers may be associated with increased harm (271). However, anti-hypertensives, anti-platelets, and statins are often stopped whilst patients are critically unwell and requiring inotropic support, and not re-started until ICU discharge, and we did not collect information regarding whether cardiac medication was stopped or continued during the ICU admission. It is interesting to note that observational research suggests that patients on statins before their ICU admission (usually for CVD) appear to have improved outcomes when these are continued rather than stopped at ICU admission (272, 273). Our data provide a possible mechanism of this benefit through protection against ischaemic events, but further research would be required to investigate this.

The biggest limitation of this study is that we were unable to differentiate between ischaemic intra-coronary plaque rupture or oxygen supply-demand imbalance, and inflammatory troponin leak other than using surrogate markers of ischaemia and inflammation. Imaging in these patients in clinical practice is limited to bedside transthoracic echocardiography, which is unable to pick up the nuances of small troponin rises. Cardiac angiography involves transferring the unstable patient out of the ICU environment, and also requires anticoagulation whether or not any intervention is performed which carries additional risk. Angiography will show coronary artery plaque rupture, but not supply-demand imbalance. CT coronary angiography will show the burden of atherosclerotic disease, and Cardiac Magnetic Resonance may well be able to identify localised versus generalised acute myocardial injury (looking at T2 oedema) and is discussed further in the future directions chapter. However, these also pose risk to the patient, and are very much a research tool at present for patients who are critically unwell.

5.5.2. Interpretation and comparison with other studies

5.5.2.1. Baseline Characteristics

The overall prevalence of cardiovascular disease in the ICUs where we screened was 23.4%. This is lower than previous UK studies (95, 155) which has implications for recruitment for future studies based in this population. This varied considerably between units, which may partly be explained by the different case-mix that presents to these centres. Lothian, Salford and Brighton all contain tertiary neurosurgical critical care beds, and these patients are frequently younger with fewer comorbidities than general ICU patients, which may explain their

low prevalence rates. The patients we recruited were older than general patients in ICU (median approximately 65 years), and predominantly male, in line with other studies (274, 275). Few patients had severe APACHE comorbidities, however, three-quarters of our patients had at least one comorbidity as measured by the functional comorbidity index (FCI) (259). This is in part due to the fact that previous myocardial infarction, angina, congestive heart failure, Stroke or TIA, Peripheral vascular disease, and diabetes are elements of the FCI and also formed our inclusion criteria, FCI has a strong association with the physical function of patients, compared with APACHE comorbidities which are designed to predict mortality.

We were able to process routine TnI samples using the high sensitivity assay for 273 out of 279 patients, from blood samples that had already been taken for clinical purposes. We found that all patients had detectable TnI at admission to ICU. Studies have also found detectable TnI in patients with stable coronary artery disease and congestive cardiac failure in the community (188, 189, 195), although studies have not looked at patients with other vascular disease including cerebrovascular disease, peripheral vascular disease, or the elderly with cardiovascular risk factors. There was a wide range of TnI concentrations at presentation to ICU, reflecting the wide range of patients, presenting diagnoses, and stages of critical illness. Admission TnI was a predictor of peak TnI but this was in part because the admission TnI was the peak TnI for approximately a third of patients. Peak TnI occurred most frequently within the first three days of ICU admission. Patients had a distinct rise and fall in TnI concentrations, reflecting an acute injury rather than chronically raised TnI. There was a distinctive main peak, with preceding and/or subsequent further smaller peaks. Studies of TnI post primary angioplasty for ST elevation myocardial infarction have shown a log linear decrease in TnI concentrations, without a subsequent peak (237), and no impact from renal impairment. This suggests that subsequent TnI peaks seen in our population may be due to an ongoing or new insult. The majority of patients who died in ICU also had a fall in TnI concentrations after the initial peak. This suggests that there was a specific stimulus for TnI release.

5.5.2.2. Objective 1: To determine the incidence of myocardial infarction and myocardial injury as defined by the Third Universal Definition of myocardial infarction.

The diagnosis of myocardial infarction outwith the critical care environment frequently relies on the patient reporting symptoms. Approximately half of our cohort were ventilated on the day of admission to ICU and in the 24 hours preceding peak TnI. We know from other studies that the prevalence of delirium in ICU varies from 45% to 87% (276). Most other patients would be receiving analgesics and many would have distracting symptoms from their concurrent primary admission diagnosis. This means that a diagnosis of myocardial infarction in ICU that relies on patients reporting symptoms of myocardial ischaemia is likely to miss many patients who are unable to communicate.

The ECG is also central to the diagnosis of myocardial infarction according to the Third Universal Definition, however its value is reduced significantly if its interpretation is unreliable. All our ECGs were interpreted by cardiologists blinded to clinical details, which other studies have found resulted in higher agreement compared with non-cardiologists (277).The overall agreement for ECGs between our cardiologists varied dependent on the abnormality present. There was fair to good agreement for specific ECG abnormalities including ST elevation, and ST depression and poor to fair for T wave inversion. These results are similar to other studies, which have found moderate inter-observer agreement (81, 232). However, we found excellent agreement for the presence or absence of dynamic changes consistent with ischaemia across each patient's ECGs. This is of particular

relevance in cardiovascular patients in ICU, who we have shown frequently have abnormal baseline ECGs, and it is the change in appearance of the ECG, rather than the specific abnormality which may reflect underlying regional myocardial ischaemia. We found that 91 patients had an ECG abnormality consistent with potential ECG ischaemia (ST elevation, ST depression, T wave inversion, Q waves, RBBB) at presentation to ICU, however in 48% of these patients, there were no changes in the ECG during the first five days of their admission. This shows the importance of serial ECGs in this population of patients with CVD, rather than reliance on a single ECG. The use of ischaemia rather than specific ECG abnormalities has resulted in a highly reliable assessment of the ECG. These are important points for clinical practice. Unless sequential changes are examined there is the potential to overdiagnose infarction by approximately twofold. In contrast, in the absence of repeated systematic ECG analysis in patients with co-existing CVD (i.e. current practice), the pick-up by clinicians was extremely low.

We used the latest guidelines for the diagnostic threshold for TnI using the highly sensitive assay. These cut-offs (16ng/l for women and 34ng/l for men) are considerably lower than previous thresholds (40 or 50ng/l dependent on the coefficient of variation at the local site), but have been shown to increase the identification of those at high risk of re-infarction and death in patients presenting with suspected acute coronary syndrome, particularly in women (170). We found that 69.6% of patients had a dynamic rise and fall pattern in TnI over the sex-specific diagnostic threshold. Restriction to five times the upper reference limit, in line with guidance for TnI elevation after percutaneous intervention, reduced this to 43.6%, with Infarction in 18.3% of patients. Previous ICU studies which were not restricted to patients with co-existing CVD have found rates of myocardial infarction between 14-36% (82, 95), and that patients with infarction had higher hospital mortality than patients with isolated elevated troponin, or no troponin elevation. Lim et al made the important point that the associated mortality was similar irrespective of whether the events were recognised or not (229). This suggests that intervention may not necessarily be beneficial, but it may also be that only the more severe cases of Infarction were recognised clinically. Lim's cohort comprised general ICU patients without co-existing CVD, and the risk-benefit ratio for cardiological intervention will have been different in this cohort.

Dynamic changes consistent with ischaemia on the ECG were associated with a peak TnI under the diagnostic threshold in only eight patients. These were predominantly T wave inversion, which in our sub-study analysis for ECG agreement received only slight to fair (kappa=0.138 for admission ECG, 0.223 for subsequent ECGs) agreement. Removal of T wave inversion from the diagnostic criteria for our sensitivity analysis had minimal impact on the relationship between ischaemia and peak TnI or ischaemia and 6 month mortality, and led to a clearer distinction between Injury and Infarction for the association with 6 month mortality. Given the poor reliability of T wave inversion, it is reasonable to suggest that dynamic ECG changes consistent with myocardial infarction in this population should not include isolated T wave changes.

5.5.2.3. Objective 2: To explore the duration of TnI elevation above baseline with respect to mechanism of injury (cardiomyocyte necrosis vs reversible ischaemia).

When we restricted our analyses to patients with TnI elevation above the diagnostic threshold, we found that the longest duration of TnI elevation was associated with higher severity of illness scores at presentation, and higher six month mortality. TnI fell to baseline/less than 20% of the peak in half of our patients within two days of the peak value, and this increased to 60-65% within three days and 89% within four days. However, we found no

difference in the duration of TnI elevation between patients diagnosed with Injury and Infarction. The true half-life of TnI is less than two hours and previous studies have found that the half-life of troponin clearance was significantly shorter in patients with non-Q wave infarcts compared to Q wave infarcts. Persistent elevation on day three or four represents degradation of the contractile elements, which is a hallmark of irreversible cell injury (278). The higher six month mortality in the patients with the longest duration of TnI elevation may be consistent with this irreversible myocardial cell injury. Some of our patients had several rise and fall peaks, rather than a single event, which may have made the duration of elevation harder to interpret.

5.5.2.4. Objective 3: To explore the relationship between TnI and biomarkers representing global inflammation (C-Reactive Protein, CRP) and global ischaemia (lactate).

We assessed the relationship between TnI and global inflammation using routine repeated CRP measurements in a subgroup of our patients. These were retrieved from the same sample as the TnI, and were not taken for clinical indication. We found that the rate of rise of CRP was considerably slower than for TnI in line with previous studies (240). Patients who had raised TnI within the first five days of admission also had raised CRP. However, using a time window based on the rate of rise of CRP and TnI, the relationship disappeared and there was no discernible relationship between peak CRP and either peak TnI or myocardial injury category. This may suggest that the specific insult causing TnI rise is separate from the insult causing the rise of CRP. However, CRP is a very non-specific marker of inflammation, and three quarters of the readings were between 183-323mg/l, which makes it hard to differentiate between no inflammation vs significant inflammation.

We assessed the relationship between TnI and global ischaemia using the highest lactate reading in each 24 hour period whilst the patient was in ICU. During the first five days of ICU admission, a quarter of patients had a peak lactate classified as “High”. We found a significant association between peak TnI and lactate in the 24 hours preceding the peak, and that the lactate was highest in the patients classified as “Infarction”. This is consistent with an ischaemic insult to the myocardium. The presence of ischaemic changes on ECG was also associated with peak TnI, and this suggests that another mechanism may also be present – regional supply-demand imbalance secondary to stable or unstable coronary artery disease. 59.7% of patients with Infarction had a lactate greater than 2.0 in the 24 hours preceding the TnI peak. This is important in terms of detecting infarction that might be amenable to improving oxygen delivery, or who would potentially benefit from ACS treatment given that these patients all have pre-existing CVD.

5.5.2.5. Objective 4: To understand the incidence of significant anaemia and its management in critically ill patients with cardiovascular disease, and its relationship with TnI

There was a high prevalence of anaemia in this cohort with 58.1% of patients experiencing significant anaemia (Hb<90g/l), which was higher than for ICU cohorts comprising all-comers (43). 35% of patients received at least one unit of RBC, and the median Hb prior to transfusion was 81g/l, although there was a wide range. This was not in line with a restrictive transfusion threshold of 70g/l, and reflected the uncertainty in transfusion thresholds in patients with CVD acknowledged by recently published guidelines (76, 77, 279). Anaemia in the 24 hours preceding peak TnI was an independent predictor of peak TnI rise, supporting the hypothesis that TnI elevation was, at least in part, a result of oxygen supply-demand imbalance. The median time to significant anaemia was the third day after ICU admission, slightly later than the median time to peak TnI, however nearly half of patients had a first or repeat rise in TnI after their Hb fell below 90g/l. For many of these patients, this

TnI rise was after we stopped collecting ECGs, and we were therefore unable to differentiate Injury from Infarction. This is important to bear in mind for the design of a blood transfusion trial with myocardial injury as an end-point.

We know anaemia is more prevalent in patients with greater illness severity and organ failure. This is likely in part because of the marrow suppressive effects of inflammation and possibly lower red cell survival. The fact that anaemia remained associated after adjustment for these factors in the analysis is consistent that it may have a causative link, as this is biologically plausible based on known coronary physiology. Furthermore, the significant interaction between SOFA and Hb support the hypothesis that the impact from anaemia was greater in patients who were more sick. Anaemia is likely to be only a part of a multifactorial causative pathway, but the data support Hb potentially having a causal role and support the hypothesis for intervention to treat anaemia for this outcome.

5.5.2.6. Objective 5: To determine the independent variables associated with TnI elevation.
The most significant predictor of peak TnI was severity of illness. However, the other significant predictors were anaemia and lactate. These also both potentially reflect an inadequate oxygen supply-demand balance.

The daily SOFA score in the preceding 24 hours was strongly associated with peak TnI. In the univariable analysis, the renal component correlated most closely with peak TnI which may be as a result of both inflammatory and ischaemic elements. There have been a number of studies that have looked at troponin in patients with sepsis, and have found that troponin elevation is a predictor of mortality even in the absence of cardiovascular disease (196, 197). This mechanism is unclear but may include direct cardiac myotoxic effects of endotoxins, cytokines (203) or reactive oxygen radicals (197).This is supported by in vitro studies showing that TNF alpha and interleukin 1-beta lead to reduced contractility of cardiomyocytes (280). However Landesberg et al found no in vivo correlation between inflammatory cytokines and systolic or diastolic myocardial dysfunction in severe sepsis or septic shock (205).

The association between SOFA and peak TnI is strong, a key consideration in the assessment of observational associations. It is biologically plausible as organ failure in acute illness results from a combination of ischaemic and inflammatory injury, both of which result in myocardial injury that will be reflected by magnitude of TnI release. It follows that causes of worse SOFA also cause worse myocardial injury based on known biological mechanisms, supporting association by concurrent relation to other variables/mechanisms. There was a correlation with the CVS element of SOFA. It is possible that myocardial injury is in part causal for worse SOFA through worse cardiovascular function. It is also possible that worse SOFA is in part causal for greater myocardial injury, for example through greater cardiovascular stress, or consequences of metabolic disturbance/renal failure. However, we cannot prove the direction of causality in this study design.

The stronger signals of association, after biologically logical adjustment for time differences to infer causality, were more supportive of a major causal relationship with ischaemic mechanisms (lactate) than a predominantly inflammation mediated mechanism (CRP). CRP is not known to be increased by ischaemia per se. Lactate is classically a marker of ischaemia, although probably also increased via inflammatory mechanisms, such as mitochondrial dysfunction. The findings do suggest that ischaemia is at least part of this process, and possibly a dominant part of the mechanism of myocardial injury in this patient group. The relatively high prevalence of

dynamic ECG changes suggesting significant areas of myocardial necrosis supports this conjecture as an argument against this being a mainly inflammatory phenomenon.

5.5.2.7. Objective 6: To explore whether myocardial injury has an independent association with the outcomes of critically ill patients with CVD.

Patients with Infarction and Injury had considerably higher rates of mortality at ICU, hospital and up to six months compared to patients with No Injury. This is consistent with the published literature in both perioperative and critical care, where TnI is associated with increased 30 day mortality (95, 97, 160, 230, 231, 281). When we stratified our univariable analyses of myocardial injury by severity of illness, we found that in sicker patients the Injury and Infarction groups had similar survival, with both being significantly lower than the No Injury group. In less sick patients, in contrast, the Injury group had survival similar to the No Injury group, whereas those with evidence of Infarction had significantly worse survival. Our sensitivity analyses suggested that if the patient had an ECG based ischaemic event this was the dominant issue rather than the TnI value (accepting the loss of power with the binary variable), whereas if the patient only had TnI elevation then the magnitude of the elevation was more closely related to ultimate mortality. This supports the 'construct' of including ECG change as a key part of diagnosis in the population. This suggests that myocardial infarction may contribute more to survival in less severely ill patients than in those with greater illness severity where the overall picture decreases the relative contribution of infarction to the outcome. It follows that it would be important to include these less severely ill patients in any trial which aims to reduce the incidence of myocardial infarction.

5.5.3. Generalisability

This study took place in patients with known CVD, or risk factors for CVD. It can therefore not be generalised to an ICU population without CVD. These patients by definition have no atherosclerotic disease in their coronary arteries, and their myocardium is therefore potentially less vulnerable to oxygen supply imbalance. These patients may have different causes of TnI release, and the inflammatory component may well play a larger part. We excluded patients with acute intracerebral pathology, and a future study should be performed in these patients to look at the dynamics of TnI release and its implications.

However, we had a broad range of patients with CVD admitted to general ICUs in both teaching and district general hospitals. Both surgical and non-surgical populations were well represented in this cohort, and we believe this study is generalisable to patients with co-existing CVD presenting to general ICUs with non-cardiac diagnoses.

5.5.4. Potential future directions

It is important to separate the use of troponin (I or T) as a prognostic marker, and the use of troponin as part of the diagnosis of myocardial infarction.

5.5.4.1. TnI as a prognostic marker

The main predictors of peak TnI were severity of illness, dynamic changes on ECG consistent with ischaemia, anaemia and lactate. Although it would be difficult to alter how sick patients are when they present to the ICU, severity of illness is made up of a number of physiological parameters including heart rate and blood pressure. It is feasible that targeting physiological parameters that contribute to severity of illness scores, and impact on

myocardial oxygen supply-demand imbalance may improve this imbalance. This may then reduce the incidence of myocardial infarction, or lessen its impact, and improve other important outcomes including mortality and patient reported outcome measures such as quality of life. A protocol for a blood transfusion threshold trial in critically ill patients with coexisting cardiovascular disease and evidence of acute myocardial injury is presented in the next chapter.

5.5.4.2. Diagnosis of myocardial infarction

We believe we have identified a cohort of patients in whom the likely cause of troponin elevation was a regional Type I or II Myocardial Infarction. In this population, 69% of patients had a biomarker rise and fall consistent with myocardial Injury. 24% (32% of those with rise and fall in biomarker) had dynamic ECG changes consistent with type I or type II myocardial Infarction when systematically screened according to the Third Universal Definition. Very few were diagnosed clinically. The suggestion from the data is that these events may have an important relationship to mortality, especially in those with less severe illness, in whom proportionate contribution to outcome may be greater.

In those patients in whom the likely cause is ischaemic, treatment must first be directed to prevent or treat the non-cardiac complications such as massive bleeding, hypotension or sepsis. Although effective interventions are unclear, clinical implications could include:

1. Continuing or starting cardiac mediations. Nearly all patients with coronary artery disease in this cohort were already on secondary cardiac medication prophylaxis (aspirin, clopidogrel, statin), so there is little scope for starting these in this patient cohort. However, we did not study whether these drugs were continued or stopped during patients' ICU stay, and this would be important to collect in future studies. It may be that in a high risk cohort, the risks of continuing these medications during critical illness (bleeding, ulceration, liver dysfunction) are outweighed by the benefits of reduced myocardial injury and infarction.
2. Ensuring secondary prophylaxis is re-established as early as possible during care and among survivor populations if it is stopped during the acute phase for other reasons.
3. Considering more active rate control or other strategies to improve myocardial perfusion
4. Correcting and tolerating less anaemia to improve oxygen content
5. In selected cases considering coronary imaging and interventions either during or after the acute phase of illness. The management of patients with primary coronary artery plaque rupture is different from patients with oxygen supply-demand imbalance, and further research should be directed at selecting out the group who may benefit from invasive angiography.

Until we can target those patients who have TnI release as a result of ischaemia, with modifiable coronary artery disease, strategies to treat all patients with TnI elevation are unlikely to prove successful, and the risks may outweigh the benefits. Future imaging studies based in CT Coronary Angiography (CTCA) and Cardiac Magnetic Resonance (CMR) in this patient population may help us to differentiate between patients who have regional myocardial injury (due to either Type I MI or Type II MI), compared with global myocardial injury (as a result of global myocardial inflammation). This is discussed further in the Future Directions section of Chapter 8.

5.5.5. Conclusion

In conclusion, we have used a systematic approach to the diagnosis of myocardial infarction according to the Third Universal Definition, which is reproducible and can be repeated in future studies and trials. More than two thirds of our patients experienced a troponin elevation above the sex specific diagnostic threshold, and approximately a quarter of patients were diagnosed with myocardial infarction. The admission ECG was abnormal in 35.6% of patients, and of these patients, 51.7% of patients experienced dynamic ischaemic changes. For patients with no potential signs of ischaemia on ECG at admission, 18.5% of patients experienced dynamic ECG changes. Agreement for T wave inversion was poor between cardiologists, and exclusion of isolated T wave inversion from the diagnosis of ischaemia identified a higher risk group of patients. In this patient population, we found that predictors representing myocardial or global ischaemia were independent predictors of peak TnI, supporting our hypothesis that TnI in critically ill patients with co-existing cardiovascular disease is at least in part due to myocardial oxygen supply-demand imbalance. These patients with regional myocardial infarction as evidenced by regional ECG changes consistent with ischaemia, and TnI elevation are a high risk group in whom further investigation and consideration of treatment for ACS may potentially be beneficial. We have identified important physiological parameters such as haemoglobin concentration, heart rate control, or mean arterial pressure that may be manipulated to improve the myocardial oxygen supply-demand balance.

6. The addition of TnI to the APACHE II model in the prediction of hospital mortality for patients with co-existing CVD

6.1. Introduction

In Chapter 3, we discussed the addition of TnI (taken within 24hours of ICU admission) to the APACHE II model in the prediction of hospital mortality for all patients admitted to Glasgow Royal Infirmary between January 2010 – June 2014. We found that TnI (using the Abbott ARCHITECT Stat TnI assay, lower limit of reporting 0.04mcg/l) was an independent predictor of hospital mortality (OR per doubling of TnI 1.16, 95% CI 1.13 to 1.20, p<0.001), and correlated most highly with the acute physiological score (APS) component of APACHE II. The addition of TnI to APACHE II did not improve the risk prediction model.

We also found that TnI was a significant predictor of hospital mortality when the dataset was restricted to patients with co-existing severe cardiac disease (New York Heart Association IV) (OR 1.20, 95%CI 1·00 to 1.49). This was a very small subset (n=49), and we wished to explore whether this relationship persisted in a larger population of patients with less severe cardiovascular disease (CVD).

In Chapter 4 we found that dynamic ischaemic changes on ECG were independently associated with higher peak TnI, and that the Infarction category (dynamic ischaemic ECG changes plus a rise and/or fall of TnI above the sex-specific diagnostic threshold) had higher mortality and longer ventilation time. The association between Infarction and 6 month mortality appeared to be affected by the severity of illness score – the contribution to mortality from Infarction was proportionately greater in less severely ill patients, although this analysis was underpowered.

6.2. Hypotheses

1. TnI taken within 24 hours of ICU admission is a stronger predictor of hospital mortality in patients with co-existing CVD compared to patients with no CVD.
2. TnI combined with ECG information will have a greater association with hospital mortality than TnI alone.

6.3. Methods

6.3.1. Hypothesis 1: TnI taken within 24 hours of ICU admission is a stronger predictor of hospital mortality in patients with co-existing CVD compared to patients with no CVD.

We repeated the methods used for the Glasgow dataset (Chapter 3), to examine whether TnI taken within 24 hours of ICU admission was an independent predictor of hospital mortality in patients with cardiovascular disease, and whether it improved the APACHE II model.

We restricted our dataset to the first 24 hours after ICU admission. We mapped APACHE III (Scotland) and ICNARC (England) diagnoses to APACHE II. We used the highly sensitive TnI assay, and kept in the additional values of TnI<0.04mg/l that were below the lower limit of reporting for the ARCHITECT STAT TnI assay that we used in the Glasgow analysis. We continued to categorise TnI>0.04mg/l as “positive”, and

TnI=<0.04mg/l as "negative". Subsequent methods are as per Chapter 3. We did not collect the raw components of the APACHE II Score, and were unable to look at correlation of TnI with each component.

6.3.2. Hypothesis 2: TnI combined with ECG information will have a greater association with hospital mortality than TnI alone.

We restricted our dataset to the first 24 hours after ICU admission. We were therefore unable to comment on the dynamic nature of ECG changes in this population, as each patient only had one ECG in the first 24 hours. We therefore used "potentially ischaemic ECG" as our category: patients who had ST elevation, ST depression, T wave inversion, Q waves or RBBB on their admission ECG.

6.4. Results

6.4.1. Hypothesis 1: TnI taken within 24 hours of ICU admission is a stronger predictor of hospital mortality in patients with co-existing CVD compared to patients with no CVD.

Hospital mortality was 30.4% (n=46) for TnI positive patients, compared with 10.5% (n=13) for TnI negative patients. There was a significant univariable association between TnI and hospital mortality (Figure 70), odds ratio per doubling of TnI 1.18 (85% CI 1.07, 1.31, p<0.001). After adjustment for the APACHE II model components, TnI remained an independent predictor of hospital mortality, but the magnitude of prediction was reduced (OR 1.13 (1.01, 1.25, p=0.034)).

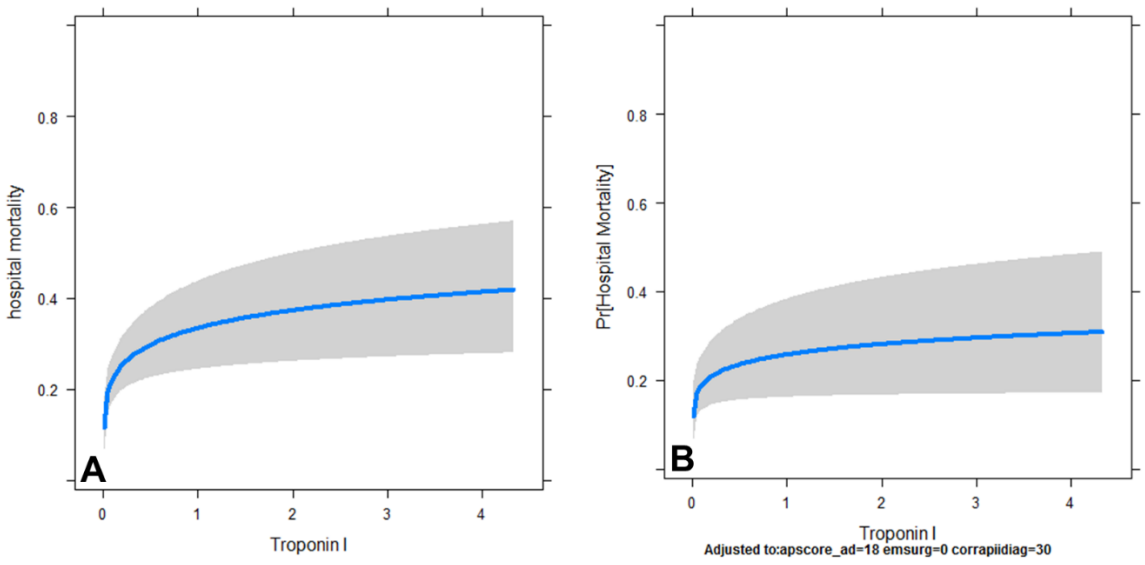

Figure 70: A. Univariable association between admission Troponin I (10 (3.7%) admission TnI>4.5mg/l, therefore not depicted) and actual hospital mortality. Odds Ratio per doubling of admission TnI 1.18 (95% CI 1.07, 1.31, p<0.001). B: Multivariable association between admission TnI (mg/l) and hospital mortality once added to the APACHE II model. OR TnI 1.13 (1.01, 1.25, p=0.034).

6.4.1.1. Addition of TnI to the APACHE II risk prediction model

The variables comprising the APACHE II model applied to the TROPICCAL dataset resulted in a c-index of 0.724 (95% CI 0.650, 0.797). The addition of TnI as a logarithmic term did not improve the discriminatory power of the model (p=0.453, c-index 0.735 (95% CI 0.665, 0.805)), nor improve other assessments of model performance (Table 39).

Table 39: Model Comparison for TROPICCAL dataset. APACHE II vs APACHE II + admission TnI. AUC: Area under curve (concordance-index), AIC: Akaike Information Criterion, p value: DeLong's test for two correlated ROC curves. OR: for doubling of TnI

	APACHE	**APACHE + TnI**	**P value**
AUC (95% CI)	0.777	0.792	0.394
	0.709 to 0.845	0.727 to 0.857	
AIC	264.04	278.64	
R2	0.228	0.247	
Brier score	0.147	0.142	
Odds ratio for TnI			
Univariable TnI	1.20 (95%CI 1.07, 1.34)		<0.001
TnI in APACHE model	1.15 (95%CI 1.01, 1.30)		0.032

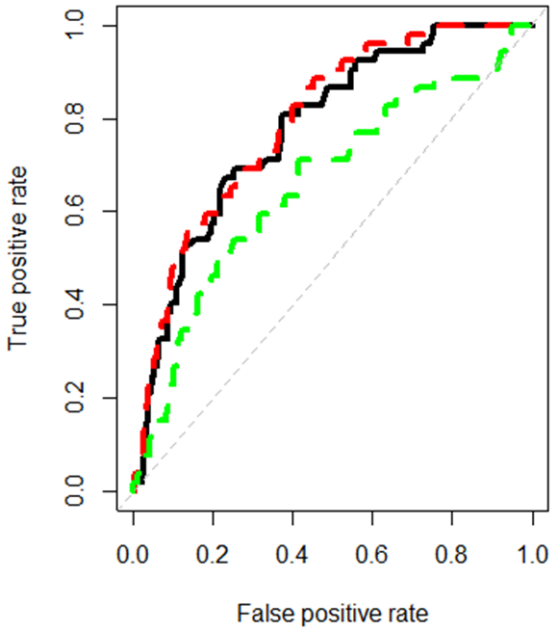

Figure 71: Receiver Operator Characteristic Curves: APACHE (black, c-index 0.777 (95% CI 0.650, 0.797), APACHE + TnI (red, c-index 0.792 (95% CI 0.665, 0.805), TnI (green, cindex 0.660 (95% CI 0.572, 0.748). difference between APACHE vs APACHE + TnI ROC curves p=0.394

6.4.2. Hypothesis 2: TnI combined with ECG information will have a greater association with hospital mortality than TnI alone.

There was no improvement in the discrimination of the model, or other assessments of model performance with the addition of ECG information at baseline (Figure 72).

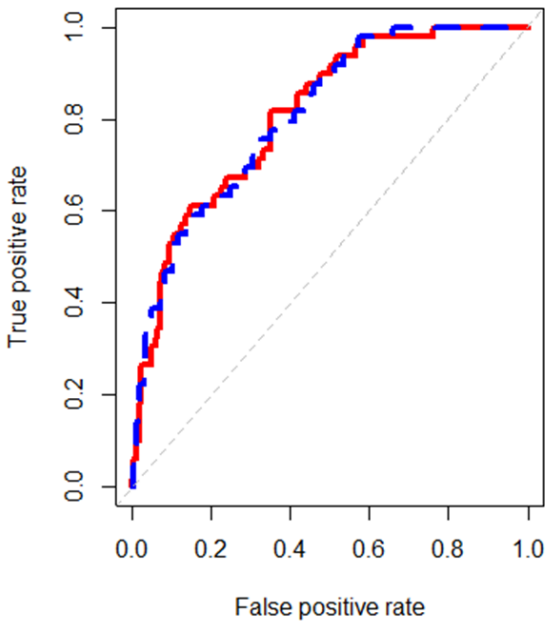

Figure 72: Receiver Operator Characteristics curve (n=217): Red: APACHE + TnI (c-index 0.809, 95% CI 0.745 to 0.834), Blue: APACHE + TnI + "potential ischaemia" on admission ECG (c-index 0.809, 95% CI 0.745 to 0.873), p=0.986

6.5. Discussion

TnI taken within 24 hours of ICU admission was associated with hospital mortality in patients with co-existing CVD. This association persisted, but was attenuated after adjustment for severity of illness and age/comorbidity as measured by APACHE II. The point estimate for the association of TnI with hospital mortality in patients with CVD (OR 1.20 (95%CI 1.07, 1.34)) was considerably greater than in the general ICU population (OR 1.05, 95% CI 1.01 to 1.09, p=0.003). The sample size was small, which was reflected in the precision of the confidence intervals, and this has created uncertainty about the conclusions and the difference in associations between the groups.

TnI did not significantly improve the predictive ability of the APACHE II model, or the model statistics. The addition of ECG changes at baseline did not improve the discriminatory ability of the model. This reflects the limitations of using admission (rather than peak) TnI, and a single admission ECG. In our main analysis of TROPICCAL we found that 35.7% (n=91) of these admission ECGs were abnormal with changes associated with potential ischaemia, however, we found that approximately half of these patients had no subsequent dynamic changes. We also found that 36.4% of patients who had dynamic ECG changes had no signs of ischaemia on their ICU admission ECG. These support the need for assessment of myocardial injury over time instead of simply at a fixed point in critically ill patients with cardiovascular disease.

The TROPICCAL cohort comprised patients with CVD, whereas the Glasgow cohort consisted of all patients admitted to ICU. The Glasgow cohort contained 49 patients with significant CVD (NYHA IV), but is likely to have had a higher proportion of patients with less severe CVD embedded in the cohort that we were unable to detect with the data that we had. This may have diluted the differences between the groups but to an uncertain

degree. The TROPICCAL cohort was older (mean age 72yrs) than the Glasgow cohort (mean age 60yrs), but severity of illness at presentation was similar (APACHE II: 18 vs 20). Hospital mortality for patients who had TnI taken in Glasgow was higher (25.5%, Table 6) than TROPICCAL (21.1%, Table 25), and the association between "negative" TnI and low hospital mortality was evident in both groups (Glasgow TnI "negative" hospital mortality 14.6% vs "positive" 37.3%; TROPICCAL TnI "negative" hospital mortality 9.5% vs "positive" 26.6%).

In conclusion, we have found an independent association between TnI taken within 24 hours of ICU admission and hospital mortality in both the overall ICU population, and those specifically with co-existing CVD. The point estimate for the odds ratio for hospital mortality was considerably higher for patients with CVD, although the smaller dataset reduced the precision of this estimate. The inclusion of a single routine ECG at admission did not improve the model. This highlights the limitations of using ICU admission as time zero for critically ill patients with CVD. Assessment of myocardial injury in these patients should take place over time, with repeated measurements to detect the peak and trend of TnI, and assessment of dynamic changes on ECGs.

7. Points to consider for a blood transfusion trial in critically ill patients with cardiovascular disease.

The standard transfusion threshold for young non-comorbid critically ill patients is 70g/l (76, 282). However, for patients with co-existing cardiovascular disease there is uncertainty regarding the optimum transfusion threshold. In Chapter 3 we showed that patients with co-existing CVD randomised to a restrictive transfusion threshold in a blood transfusion trial had a 78% higher risk of new Acute Coronary Syndrome compared to patients who were randomised to a liberal transfusion threshold (91). Both restrictive and liberal thresholds varied between trials, and in fact overlapped. Furthermore, the diagnostic criteria also varied between trials, and the clinicians/investigators making the diagnosis of ACS were not always blinded to the threshold arm, introducing the significant risk of ascertainment bias.

When we looked prospectively at myocardial injury in critically ill patients with CVD in Chapter 5, we found that anaemia was common, and that there was considerable variation in transfusion thresholds for these patients, reflecting the uncertainty in current guidelines. We also found that there was a significant association between anaemia and peak TnI level, and between anaemia and mortality at six months.

20-30% of patients admitted to ICU in the UK have co-existing CVD (95, 155). The uncertainty in current guidelines, along with the work we have performed in this thesis suggests that a blood transfusion trial in this population is warranted. In this chapter, I will describe the design of a trial in this population and discuss a number of issues surrounding this.

7.1. Trial design

There is a spectrum of severity of both critical illness and CVD, and it follows that the balance of risks and benefits of transfusion may change along this spectrum. A pragmatic RCT of restrictive versus liberal blood transfusion thresholds in patients with co-existing acute and chronic cardiovascular disease, with mortality as a primary outcome is likely to encounter practice misalignment, where inclusion of heterogeneous populations in trials can mask potentially divergent effects in sub-populations (154). This was demonstrated in an analysis of the TRICC (Transfusion Requirements in Critical Care) which stratified participants by presence/absence of CVD and showed that the mortality risk was greater in the liberal group for patients without CVD, and greater in the restrictive group for patients with CVD (154).

One approach could be to limit the trial population to the highest risk group, such as those with objective evidence of myocardial injury (i.e TnI above the sex-specific diagnostic threshold) at the point of recruitment. This would mean that we are focussing on the group where it is biologically most plausible that a more liberal transfusion threshold may be beneficial, and where we are most likely to see a difference in outcome between restrictive and liberal thresholds. Furthermore, as we showed in TROPICCAL, these patients have a high incidence of both Infarction and mortality which, if we were to use either as our primary outcome, would potentially make trial numbers smaller. However, this would exclude patients who could go on to develop

myocardial injury or infarction after a more prolonged exposure to significant anaemia. In addition, if there is a benefit in this high risk group, we would be unable to recommend practice for the lower risk groups, and would potentially need to carry out a subsequent trial in these groups.

Another approach could be to include the whole population, and then pre-define subgroup analyses. These groups could include patients with myocardial infarction/injury at randomisation, or the most severely unwell patients.

We could instead minimise by variables which we believe have an independent association with our primary outcome. Minimisation aims to ensure that treatment arms are balanced with respect to predefined variables as well as for the number of patients in each group. This means that any difference seen in the primary outcome is unlikely to be as a result of baseline differences in the treatment arms. Based on multivariable regression from TROPICCAL, for a primary outcome of Infarction we could potentially minimise on SOFA score, TnI and dynamic ECG ischaemia (ie Injury and/or Infarction at randomisation), and also lactate and age.

7.1.1. Adaptive trial design

An adaptive design is a clinical study design that uses accumulating data to decide how to modify aspects of the study as it continues, without undermining the validity and integrity of the trial. There are a number of advantages to using this design in our trial:

7.1.1.1. Response adaptive randomisation design

It is physiologically appealing to design a trial that individualises transfusion based on patient risk of new myocardial infarction and/or mortality. Those patients at high risk would be transfused at a higher threshold than those at lower risk. It is also appealing to then use an adaptive approach to feed participant outcomes back into the risk algorithm to guide transfusion thresholds for future patients. This modification of the randomisation algorithm would increase the probability that a patient is allocated to the most appropriate arm, reducing the risk of patient harm and potentially reducing sample size and overall trial cost. The use of MI as the primary outcome rather than longer term mortality would enable quicker feedback. In TROPICCAL, variables that had an independent association with peak TnI in the preceding 24 hours were age, SOFA score, lactate, Hb concentration (and the interaction between SOFA and Hb), and dynamic ischaemia on ECG. We could model risk in the TROPICCAL cohort to inform the initial randomisation algorithm before recruitment started.

Analysis, type I error control and sample size calculations become more complicated with this design. An additional consideration is that patients enrolling later in the study have an increased chance of receiving the superior treatment since the randomisation probability will have increased for the better treatment.

7.1.1.2. Drop-the-loser design

Drop-the-loser designs allow dropping the inferior treatment groups, and also allow adding additional arms. This could be used to identify the optimum blood transfusion thresholds. As discussed above, the choice of blood transfusion thresholds is based on clinician opinion and biological plausibility. We do not know if a liberal threshold of 90g/l is better or worse than 100g/l, and this design could enable us to investigate this further.

7.2. Trial design using the PICO format

Population: Critically ill patients with co-existing CVD whose Hb concentration falls below 90g/l

Intervention: Red blood cell transfusion

Comparator: Restrictive threshold 70g/l vs Liberal threshold 90g/l

Outcome: New events of myocardial infarction

7.3. Population

7.3.1. Inclusion and Exclusion criteria

I suggest that a trial in adult critically ill patients with CVD should follow the broad inclusion and exclusion criteria we set out for TROPICCAL (Table 40, Table 41) in order to maximise generalisability.

Table 40: Inclusion criteria for blood transfusion threshold trial in critically ill patients with co-existing CVD.

Inclusion criteria	
Chronic heart disease:	previous MI, known coronary artery disease, chronic heart failure (left/right/CCF), chronic atrial fibrillation on medication, valvular disease (not secondary to intravenous drug use).
Cerebrovascular disease:	previous Cerebrovascular Accident or Transient Ischaemic Attack
Peripheral Vascular Disease:	previous vascular surgery, under review by vascular surgeons with symptoms of ischaemia, known Abdominal Aortic Aneurysm (not secondary to connective tissue disease)
Age ≥75 years with at least one risk factor for CVD:	diabetes or hypertension on medication
AND	
Admission to Level 3 ICU or combined Level 2/3 Unit AND	
Hb level ≤90g/l	

Table 41: Exclusion criteria for blood transfusion threshold trial in critically ill patients with co-existing CVD.

Exclusion criteria
New ST elevation MI at the time of randomisation
Decision for palliative care, or not expected to survive the next 48 hours
Acute intracerebral pathology including acute ischaemic/haemorrhagic CVA, SAH, TBI
Patients who have undergone cardiac surgery in this hospital admission
Lack of informed consent (either patient, next of kin, or consultee in England)
Documented wish against transfusion
Life-threatening bleeding at time of randomisation
Age <18 years

We would exclude patients in whom the primary diagnosis for ICU admission is acute ST elevation myocardial infarction. Here the guidelines already support a more liberal transfusion threshold and clinicians may lack equipoise, and therefore not enrol patients in whom they believe a restrictive threshold would be harmful. There is a separate trial (Myocardial Ischaemia and Transfusion (MINT) Trial) in progress which aims to recruit 3500 patients with acute coronary syndrome (283). We would exclude patients with direct myocardial trauma (for example cardiac arrest, penetrating chest trauma, cardiac surgery) because myocardial injury is an important outcome in this population, and these patients will have troponin elevation and potentially ECG changes due to their trauma. This will make it hard to detect troponin elevation secondary to an oxygen supply-demand imbalance. We would also exclude patients with a primary diagnosis of acute intracerebral pathology because these patients may also have troponin elevation secondary to their intracerebral pathology. We would exclude patients who are not expected to survive the next 48 hours, or who have non-escalation or palliative treatment plans in place. This means that we would be unlikely to recruit patients who die within the first four or five days in ICU. There are many causes of early mortality in critically ill patients, and it is difficult to believe that a 10-20% difference in Hb concentration could have much impact compared with severity of presenting illness in patients who die whilst in ICU. This would reduce the impact from overwhelming severity of illness, enabling us to target our intervention in patients where there is biological plausibility that a difference in Hb concentration can affect outcome. This also reduces the competing risk of death for the outcome of Myocardial Infarction and other non-mortality outcomes. We would also exclude patients aged less than 18 years. The prevalence of CVD is extremely low in this population, and is more likely to be congenital cardiac disease which may have a different relationship with anaemia.

7.3.2. Baseline characteristics for cohort

Based on data from TROPICCAL, 23.4% of patients admitted to participating ICUs had co-existing CVD. Of these patients, 60.5% met at least one exclusion criteria (post cardiac arrest, acute intracerebral pathology, post cardiac surgery, traumatic myocardial injury, expected stay in ICU <48hrs, or age <18 years). We recruited 282 patients, and have a full dataset for 274 patients. Table 42 shows the baseline characteristics of the overall cohort, and also for patients who would be eligible for a transfusion trial of 70g/l vs 90g/l. 37.0% of patients eligible for recruitment (Hb<90g/l) would fit more than one inclusion criteria. 59.9% of the patients eligible for the trial would have co-existing cardiac disease, 14.2% would have had a previous CVA/TIA, 22.8% had a history of PVD, and 31.5% would be older than 75yrs with a history of diabetes and/or hypertension. The mean age would be 72.8 years, similar to the overall TROPICCAL cohort, and older than the mean ICU age. Approximately a quarter (24.1%) of patients would be admitted after elective major surgery, and slightly more after emergency surgery (29.0%). Nearly half (46.9%) of the patients would be emergency medical admissions. These patients would be critically unwell on admission to ICU with a median APACHE II Score of 19 (IQR 15, 24), and around half (52.5%) would be ventilated on the first day of ICU admission.

Table 42: Baseline Characteristics and outcomes for overall TROPICCAL cohort (TROPonin I in Cardiovascular patients in CriticAL care), Eligible cohort: patients with Hb≤90

Variable	**Whole cohort**		**Hb≤90g/l**	
		%		%
n	279		162	
Age mean(SD)	72.3	10.9	72.82	11.2
Male sex n(%)	200	71.7	112	69.1
CVD n(%)				
ACS	11	4	14	8.7
Chronic cardiac disease	97	35	97	59.9
CVA/TIA	32	11.6	23	14.2
PVD	47	17	37	22.8
>75, DM/BP	90	32.5	51	31.5
System diagnosis n(%)				
Respiratory	65	23.3	32	19.8
CVS	64	22.9	46	28.4
GI	84	30.1	47	29.0
Genito-urinary	31	11.1	19	11.7
other	34	12.2	18	11.1
Comorbidity				
Apache: 0	233	83.5	135	83.3
1	36	12.9	20	12.3
≥2	10	3.6	7	4.3
Type of admission n(%)				
Elect Surgical	56	20.1	39	24.1
Emerg Surgical	81	29	47	29
Non-operative	142	50.9	76	46.9
Severity of Illness in first 24h				
APII score	18	15,23	19	15, 24
SOFA score	7	5,10	6	4, 9
Vasopressors n(%)	104	37.3	67	41.9
Mech Vent n(%)	134	48.2	85	52.5
RRT n(%)	8	2.9	5	3.1
Hb admission g/l med (IQR)	111	93, 125	96	86, 112
nadir Hb	84	75, 100	77	72, 82
TnI admission ng/l med (IQR)	25	7, 56	37	13, 150
Peak TnI ng/l	104	25, 375	129	33, 512
Myocardial Injury				
None	84	30.1	43	26.5
Injury	126	45.2	73	45.1
Infarction	69	24.7	46	28.4

7.3.3. Current transfusion practice in critically ill patients with CVD

Anaemia was common in critically ill patients with CVD: There was a wide range of nadir haemoglobin (med 84g/l (IQR 75, 100). The nadir Hb in the first 10 days after ICU admission was <90g/l for 58.1% (n=162) of patients, <80g/l for 39.1% (n=109), and <70g/l for 31 patients (11.1%) Figure 73).

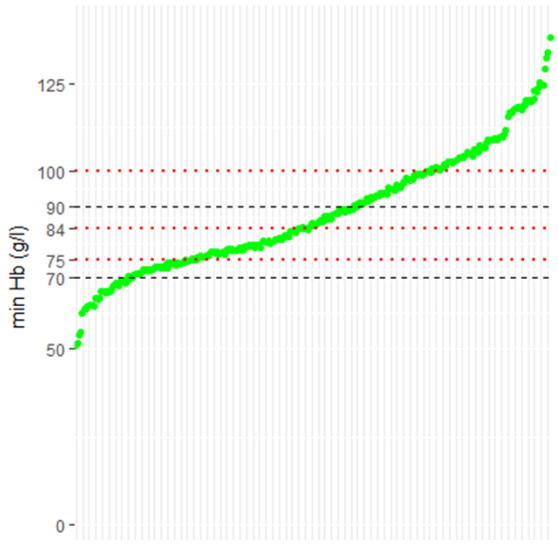

Figure 73: Nadir Hb for whole TROPICCAL cohort (n=162). Min: 51g/l, Q1 75g/l, Median 84g/l, Q3 100g/l, max 138g/l. 58.1% of patients with nadir Hb<90g/l, 39.1% <80g/l, 11.1% <70g/l

The median time to significant anaemia (≤90g/l) was the third day of ICU admission (IQR 2, 5) (Figure 74). All patients became eligible for recruitment by day seven of their ICU stay.

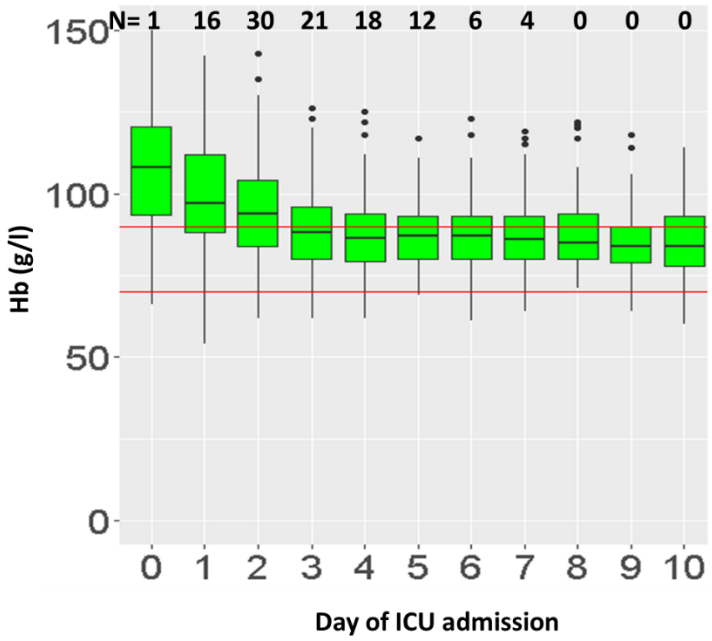

Figure 74: Distribution of Hb by day of ICU admission for patients eligible for trial (i.e Hb<90g/l during first 10 days of ICU). (Day 0=pre-ICU admission). N = number of patients whose Hb first falls to <90g/l on that day. All patients would be recruitable by day 7.

RBC transfusion was also common: 98 (35.1%) of all patients we recruited received RBC transfusion, which increased to 51.2% (n=83) when restricted to patients with nadir Hb <90g/l. There was a broad range of transfusion thresholds (Figure 75), reflecting the uncertainty acknowledged by the guidelines. The median Hb prior to transfusion was 81g/l (IQR 74, 91). When we excluded patients who were actively bleeding (patients who received three or more units of RBCs at one time point), the median Hb pre-transfusion was 79g/l (74, 88). 79 (48.8%) patients with Hb <90g/l were not transfused, 39 (35.8%) patients with Hb <80g/l (35.8%), and 4 (12.9%) patients with Hb <70g/l.

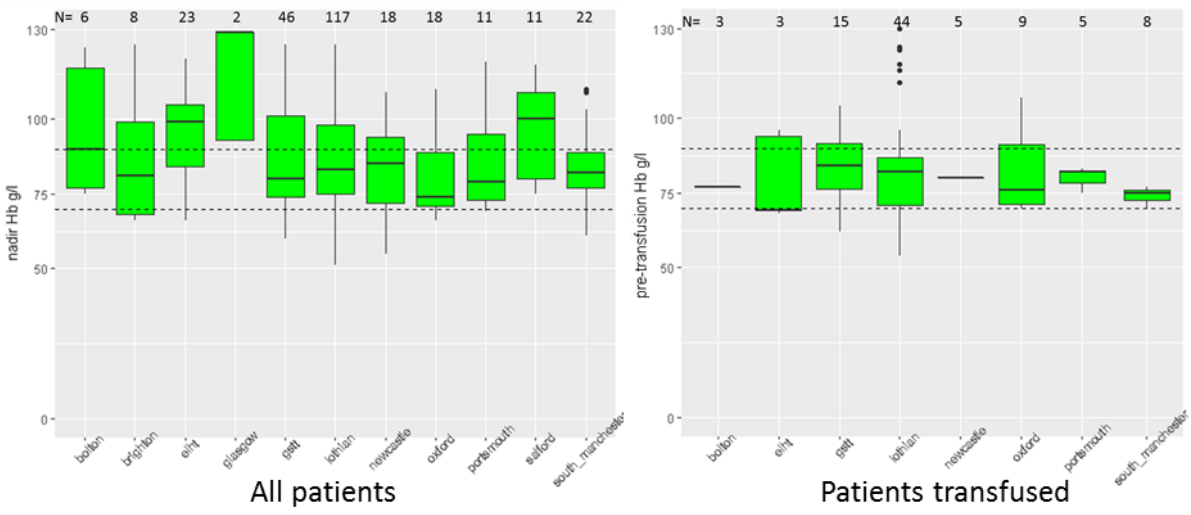

Figure 75: Left: Box-plot for nadir Hb for all patients in TROPICCAL cohort by hospital site, horizontal lines at 70g/l and 90g/l, N=number of patients recruited by site. Right: Transfusion trigger for all patients transfused in TROPICCAL cohort (no transfusions in Brighton, Glasgow or Salford), N=number of patients transfused (total n=83).

7.3.4. Myocardial injury in critically ill patients with CVD

All patients had detectable TnI, and 195 (69.9%) patients had peak TnI above the sex-specific diagnostic threshold used in the Universal definition for myocardial infarction. 66 patients with TnI above the diagnostic threshold (23.7%) had associated ECG changes consistent with ischaemia, and were classified as Myocardial Infarction. The remaining 129 patients (46.2%) with elevated TnI above the threshold had no ECG changes and were classified as Acute Myocardial Injury. Independent predictors of TnI rise in the preceding 24 hours were severity of illness (SOFA score), Hb concentration, age, lactate, and ischaemic changes on ECG.

For patients exposed to significant anaemia, 26.5% were classified as No Injury, 45.1% as Injury and 28.4% as Infarction (Table 43).

7.3.5. Outcomes for critically ill patients with CVD and significant anaemia

For patients who were exposed to significant anaemia (Hb <90g/l, n=162), approximately a quarter (26.5%) of patients did not experience myocardial injury in the first ten days after ICU admission (Table 43). Patients with Infarction and Injury had longer duration of mechanical ventilation and stay in ICU and hospital, and worse mortality up to six months compared with patients with No Injury. Infarction was an independent predictor of mortality after adjustment for severity of illness, age, comorbidity, and admission diagnosis.

Table 43: Outcomes for patients Hb≤90g/l from TROPICCAL cohort, and stratified by myocardial injury

	All Hb<90 cohort		**No Injury**		**Injury**		**Infarction**	
	n/median	%/IQR	n/median	%/IQR	n/median	%/IQR	n/median	%/IQR
n	162		43	(26.5)	73	(45.1)	46	(28.4)
MV duration med (IQR)	3	(0, 7)	2	(0,5)	3	(0,8)	6	(3,10)
ICU LOS med (IQR)	7	(4, 13)	6	(3,11)	8	(4,12)	8	(5,15)
Hosp LOS med (IQR)	21	(11, 37)	20	(11,33)	21	(12,33)	26	(11,47)

Mortality								
ICU n(%)	27	(16.7)	9.3	(9.8)	15	(20.5)	8	(17.4)
Hospital n(%)	42	(25.9)	14.0	(14.6)	20	(27.4)	16	(34.8)
90 days n(%)	47	(29)	20.9	(22.0)	22	(30.1)	16	(34.8)
6 months n(%)	55	(34)	25.6	(26.8)	26	(35.6)	17	(37.0)

7.4. Intervention

We will correct anaemia with RBC transfusion. RBC transfusion is current standard practice for correcting anaemia in critically ill patients. Oral iron therapy is unlikely to be effective due to inhibition of uptake by hepcidin, and once it is absorbed, little of it is available for erythropoiesis. Intravenous iron has not been shown to improve outcomes or reduce RBC transfusion (50, 51), although may increase Hb concentration at hospital discharge (51). Erythropoeitin has also not been shown to be effective in critical illness for reducing transfusions or generating clinically relevant increments in Hb (53).

7.5. Comparator

Previous transfusion trials have all compared restrictive with liberal transfusion thresholds, either using Hb concentration, or haematocrit. There are limitations in using Hb, as concentrations are significantly affected by fluid balance, and may not represent RBC mass. Furthermore, Hb concentrations tell us nothing about the oxygen tension in the tissues, and individual patient variation. However, reliance on symptoms of anaemia such as breathlessness or chest pain is not feasible in our critically ill patients who are often sedated and ventilated. Potential surrogate measures of tissue oxygenation, such as Near-Infrared Spectrometry and gastric tonometry, have not been validated in critically ill adults and are not in routine clinical use. On balance, Hb concentration is routinely measured in all ICUs, and is reproducible and pragmatic.

Thresholds have varied between trials (restrictive thresholds between 70-90g/l, liberal 90-113g/l (91)), and there is overlap between restrictive thresholds in some trials, and liberal thresholds in others. TROPICCAL has shown a wide variation in transfusion thresholds in critically ill patients with CVD in clinical practice (median Hb pre-transfusion 79g/l, IQR (74, 88)). This was consistent with previous UK audits which showed that around 20% of blood product use was outside guideline recommendations (284), and an analysis of the ABLE trial (Age of transfused blood in critically ill adults) which showed that the presence of CVD modified transfusion thresholds (285). The larger the difference in thresholds, the less likely it is that the treatment arms will overlap, and that a difference will be shown if it exists. It follows on that a larger threshold difference would also require fewer patients. Clinicians need equipoise however to allow patients to be enrolled in the trial, and if the restrictive threshold is too low, they may decline to enrol high-risk patients into the trial. We presented our work at the UK Critical Care Research Forum (May 2016), where there was widespread support for a restrictive threshold of 70g/l and a liberal threshold of 90g/l. These thresholds are also in line with current guidelines.

Once randomised to a liberal or restrictive transfusion threshold, patients will be maintained on this threshold until hospital discharge, or a maximum of 30 days. Literature suggests that anaemia in ICU survivors persists after hospital discharge and is still present up to six months later (43, 44). Pilot work that we have carried out in

the Royal Infirmary Edinburgh suggests that there is little active management of anaemia in general ICU survivors. We found that 21% (n=189) of general ICU survivors were discharged from hospital with Hb≤90g/l, and that only 24% of these patients were prescribed any therapy for anaemia, or had anaemia mentioned on their discharge summary. This supports a similar recent study in Oxford which found that 19% of patients received RBC transfusion post-ICU, with a mean transfusion trigger of 79g/l, and that follow-up or management of anaemia was not mentioned in any discharge summary at any time (286).

7.6. Outcomes

7.6.1. Primary Outcome

There are several feasible options for a primary outcome in a blood transfusion trial in critically ill patients with CVD.

7.6.1.1. Myocardial Infarction (MI)

MI is an appropriate outcome for a blood transfusion trial in critically ill patients with CVD at risk of myocardial injury. However, diagnosis of MI in critically ill patients is not straightforward. Diagnosis according to the Third Universal Definition of Myocardial Infarction requires a combination of patient symptoms and signs, evidence of ischaemia on ECG and a rise and/or fall pattern of cardiac biomarker (usually TnI or TnT) (94). However, in critically ill patients, standard symptoms are often masked by sedation, delirium or analgesia. Half of the eligible patients in our cohort were mechanically ventilated on the day of admission to ICU, and therefore unable to communicate symptoms. We have shown that baseline ECGs in patients with CVD are frequently abnormal (45.3%), and that ECG interpretation is difficult and inconsistent, even between expert cardiologists (good agreement for ST elevation (kappa=0.628) and RBBB (0.698); fair agreement for ST depression (0.249) and only slight agreement for T wave inversion (0.138); excellent agreement whether dynamic changes consistent with ischaemia over serial ECGs (0.890)). Based on this work, we will not include isolated dynamic T wave inversion in the criteria for Myocardial Infarction in this cohort. TnI elevation is prevalent in the critically ill, and we have shown that a TnI value below the diagnostic threshold at ICU admission does not rule out MI later on in the admission.

For this trial, our endpoint of Myocardial Infarction would be in line with current practice as follows:

after randomisation into the threshold arm and then for 30 days
≥20% dynamic rise and/or fall pattern of TnI using a highly sensitive assay over the sex-specific diagnostic threshold (16ng/l for women, 34ng/l for men)
together with at least one of the following:
symptoms of ischaemia
dynamic changes on ECG consistent with ischaemia: ST elevation, ST depression, new pathological Q waves, RBBB (NOT isolated T wave inversion)
new regional wall motion abnormality (RWMA) on echocardiography (TTE performed on clinical suspicion)
intracoronary thrombus (angiography performed on clinical suspicion)

This will require daily TnI samples, and daily ECGs for the duration of the patient's ICU stay, and then based on clinical suspicion once the patient has recovered from critical illness and is not delirious and therefore able to report symptoms.

We will also report Myocardial Injury:

after randomisation into the threshold arm

≥20% dynamic rise +/- fall pattern of TnI using a highly sensitive assay over the sex-specific diagnostic threshold (16ng/l for women, 34ng/l for men) with symptoms of ischaemia, or ischaemic changes on ECG, or RWMA on echo, or intracoronary thrombus.

There are two major points to consider with the use of Infarction as an outcome:

7.6.1.1.1. Competing risk of death

Competing risk of death is when the patient dies before experiencing the outcomes (Infarction). In TROPICCAL, 36 (12.9%) patients died in ICU. Of these, 32 patients had a rise and fall pattern of TnI before they died, and only four patients died on the day of peak TnI, suggesting that competing risk of death may be relatively low in this acute period.

7.6.1.1.2. Definition of Infarction and Injury in patients with Hb≤90g/l

There were 162 patients whose Hb fell below 90g/l. 119 (73.4%) of these patients had a peak TnI above the sex-specific diagnostic threshold during the first ten days after ICU admission (Injury n=81, Infarction n=42, Figure 76). This suggests that rates of Infarction that might be seen in a restrictive arm of a transfusion threshold in the first ten days after ICU admission, irrespective of the exposure to anaemia, would be approximately 25.9%. However, the rate of Infarction potentially temporally attributable to exposure to significant anaemia (Hb<90g/l) would be 18.5% if peak TnI occurred after exposure to anaemia (including patients where this value was the first value, or TnI was already elevated before the patient became anaemia), to as low as 7.4% for a rise >20% in TnI above the diagnostic threshold with a normal TnI preceding anaemia. Rates of "Injury+Infarction" (i.e rise and/or fall of TnI above sex-specific diagnostic threshold with or without ECG changes) potentially attributable to exposure to anaemia would be 20.4% (normal TnI preceding exposure to significant anaemia) to 48.8%

(including patients where this value was the first value, or TnI was already elevated before the patient became anaemia).

It would therefore be essential to take TnI in patients prior to Hb<90 in order to determine the direction of the TnI rise/fall. I propose that the end-point of Infarction for this trial should include all Infarctions where the peak in TnI occurs after the Hb threshold drops below 90g/l (ie including those where there are no values taken before Hb<90g/l and where TnI is lower but already elevated before Hb<90g/l). This may overestimate the rate of Infarction attributable to anaemia, but it is objective, and will miss few events.

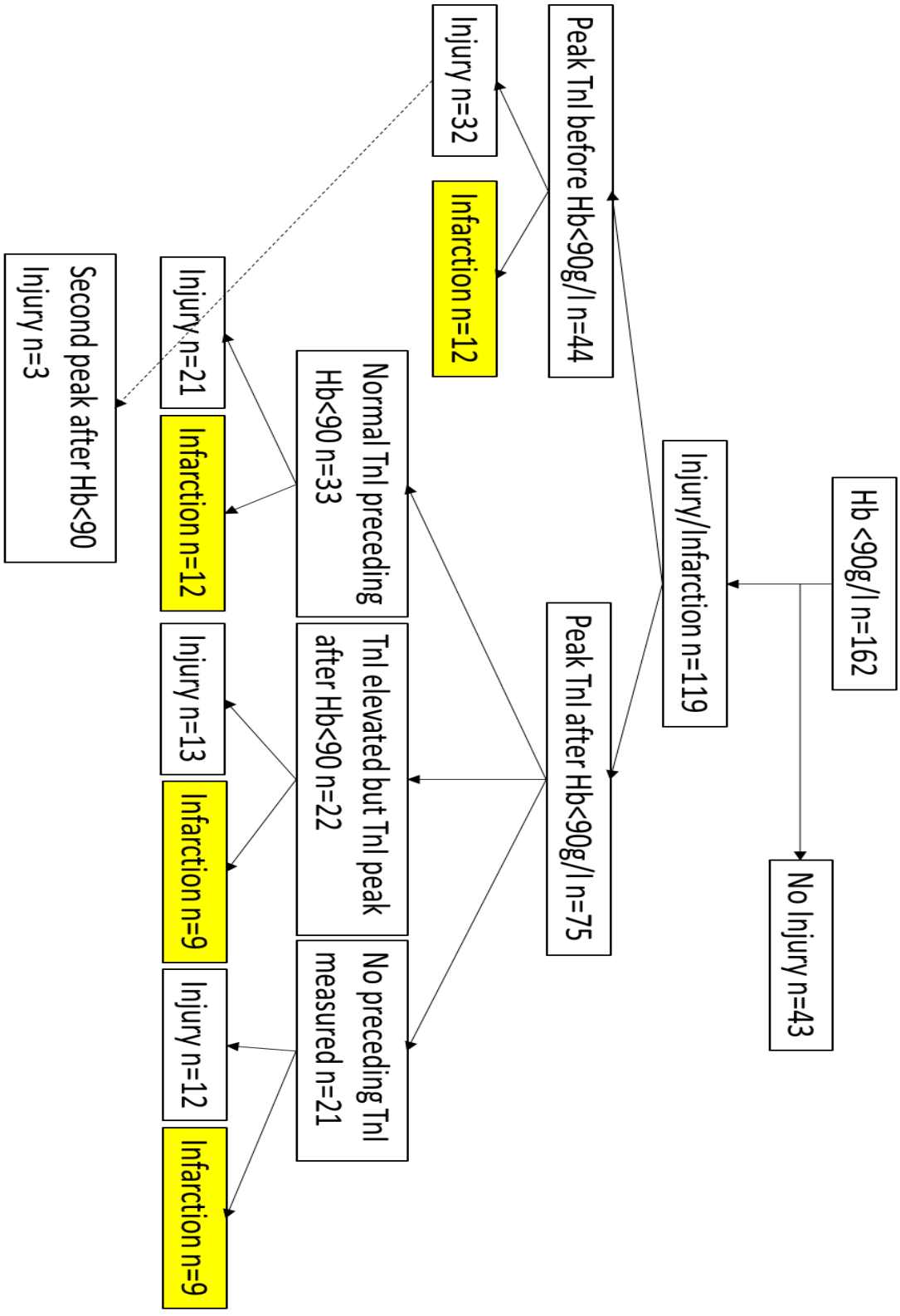

Figure 76: Potential rates of Myocardial Infarction in Restrictive arm of blood transfusion threshold arm

7.6.1.2. Mortality

The majority of blood transfusion threshold trials have used 30 day mortality as their primary outcome. However, there are many causes of early mortality in critically ill patients, and it is difficult to believe that a 10-20% difference in Hb concentration could have much impact compared with severity of presenting illness in patients who die whilst in ICU. TROPICCAL found that the median time to eligibility was three days (IQR 2, 5). We will exclude patients who are not expected to survive the next 48 hours, or who have non-escalation or palliative treatment plans in place. This means that we will be unlikely to recruit patients who die within the first four or five days in ICU. We believe that this will reduce the impact from overwhelming severity of illness, enabling us to target our intervention in patients where there is biological plausibility that a difference in Hb concentration can affect outcome. Longer term 90 day mortality, as used in the TRISS trial, will give us time to evaluate the difference between the two arms. This is a robust and clinically important outcome which is not susceptible to bias, a key consideration given that it will not be possible to blind clinicians.

There were 47 (29.0%) patients whose Hb fell below 90g/l who died within 90 days of ICU admission (Table 43).

7.6.1.3. Composite Outcome for Myocardial Infarction and 90 day mortality

In the cohort of patients with Hb<90g/l, there were 16 (9.9%) patients who were diagnosed with Infarction and died within 90 days (Table 44). 31 (19.1%) patients died without Infarction, and 30 (18.5%) patients were diagnosed with Infarction and survived beyond 90 days. The overall rate of 90 day mortality or Infarction was therefore 47.5%.

There is a range in severity in this composite outcome, where potentially death within 90 days and a diagnosis of Infarction is the most serious, followed by death without Infarction, then Infarction without death, and then no Infarction and no death. The EUROTHERM trial had an ordinal scale as their primary outcome (Glasgow Outcome Score, extended), and were able to exploit this by applying a sliding dichotomy to the analysis. This meant they were able to compare the scales in the two arms, rather than the single crude overall rate of the composite outcome. This has both clinical and statistical significance.

Table 44: Composite outcomes for Myocardial Infarction and 90 day mortality in patients with Hb<90g/l

Outcome	**n**	**%**
n	162	
90 day mortality AND Infarction	16	(9.9)
90 day mortality, NO Infarction	31	(19.1)
Infarction, NO 90 day mortality	30	(18.5)
90 day mortality OR Infarction	77	(47.5)
NO 90 day mortality or Infarction	85	(52.5)

7.6.2. Secondary Outcomes

7.6.2.1. Patient-reported outcome measures

Health status measurements quantify the extent to which a patient is impaired by the disease. Studies have consistently shown an association between anaemia and reduced Health Related Quality Of Life (HRQOL) in patients with other chronic disease such as malignancy (45) and end-stage renal disease (46). Fatigue is a commonly reported symptom amongst both anaemic patients and survivors of critical illness (47). Hb concentration between the restrictive and liberal transfusion arms remained different at hospital discharge in the RELIEVE trial, suggesting that longer term anaemia and its effect on HRQOL is potentially important for survivors (141). However, the causal relationship between anaemia and HRQOL in survivors of critical illness is not well studied. To assess HRQOL, we would use the EQ-5D validated questionnaire combines physical, social/family, emotional, and functional wellbeing. In addition we could use the 18 additional questions directed at symptoms of anaemia from the Functional Assessment of Cancer Therapy – Anemia (FACT-An, version 4) questionnaire (287). We have taken this questionnaire from the cancer literature where it has been validated to address specific quality of life concerns related to anaemia and fatigue in cancer patients. However, it is not yet validated for use in critical care, and part of our aim would be to assess its use in this critical care population, and its ability to discriminate between patients with and without anaemia, who have multiple other reasons for fatigue in their post-ICU recovery.

7.6.2.2. Cost effectiveness

In TROPICCAL, we found that patients who were transfused received a median of 3.5 units (IQR 2, 6) in the first 10 days of their ICU admission. In recent trials, even patients in the liberal transfusion threshold typically only received two or three units of RBCs (91). The cost of a unit of blood in the UK is approximately £120, but this does not take into account the complications arising from or avoided by transfusion. Evaluation of the cost-effectiveness of RBC transfusion is essential. The combination of very high hospital costs for critically ill survivors (288) and low HRQOL during the months following survivorship means that the loss of quality-adjusted life years (QALYs) is substantial following critical illness. RBC transfusion could decrease mortality, new ACS, and other complications which may impact further on quality of life, thus improving HRQOL and cost effectiveness. Alternatively a restrictive strategy could reduce the number of units transfused and any resulting complications, thus improving cost effectiveness. The Transfusion Indication Threshold Reduction (TITRe) II blood transfusion threshold in cardiac surgery trial found no clear difference in cost effectiveness between restrictive and liberal transfusion arms up to three months (289). They used the EQ-5D questionnaire for QALYs pre-operatively, and at six weeks and three months postoperatively. However, the majority of our patients will be emergency admissions, and we will be unable to obtain this baseline data. There is a recalled and a surrogate version of the EQ-5D which attempts to quantify pre-admission quality of life, either by asking the patient to remember retrospectively, or asking their close relatives. Much of the patients' post-ICU recovery will be associated with their pre-admission comorbidity, and assessment of quality of life must take this into account. Stratification approaches based on comorbidity index may be a useful means of exploring effects on quality of life and costs within each stratum. We will collect health resource use date during the acute hospital stay and via questionnaire up to six months.

7.6.2.3. Duration of mechanical ventilation

Patients with CVD may develop myocardial ischaemia associated with the increased sympathetic activation associated with difficulty weaning from the ventilator. There is biological plausibility that RBC transfusion prior to weaning may reduce the incidence of ACS, and studies have suggested an association between anaemia and failure to wean, and between RBC transfusion and reduction in the work of breathing (75). However, this is a difficult end-point to interpret due to the competing risk of death. If transfusion of RBCs moves a patient from the ICU mortality group to the survivor group, this will result in an increased duration of mechanical ventilation and potentially misleading conclusions. We will therefore use Ventilator Free Days (VFDs), defined arbitrarily as the number of days between successful weaning from mechanical ventilation and day 28 after study enrolment (290). Here, if the patient dies or the patient requires mechanical ventilation for 28 days or more, the VFDs are zero.

7.7. Sample size calculation

Calculation of the sample size requires a decision to be made regarding the minimal clinically important difference (MCID). This can be defined as the "smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate, in the absence of troublesome side effects and excessive cost, a change in the patient's management" (Jaeschke 1989). This is informed by clinical practice, not statistics. A smaller difference requires larger numbers. An absolute risk reduction (ARR) of 5% represents a biologically plausible treatment effect, and is consistent with a number needed to treat of 20. There is a range of MCID used in recent blood transfusion trials. 5% was used in the ABLE trial. The TRISS trial used a 9% ARR, the HEMOTION trial will use 10%, TITRe2 used 6%, FOCUS used 7%.

7.7.1. Primary outcome: Myocardial Infarction

Rates of Infarction that might be seen in a restrictive arm of a transfusion threshold in the first ten days after ICU admission, irrespective of the exposure to anaemia, would be approximately 25.9%. However, the rate of Infarction potentially temporally attributable to exposure to significant anaemia (Hb<90g/l) would be 18.5% if peak TnI occurred after exposure to anaemia (including patients where this value was the first value, or TnI was already elevated before the patient became anaemia), to as low as 7.4% for a rise >20% in TnI above the diagnostic threshold with a normal TnI preceding anaemia.

Based on a potential rate of 18.5% in the restrictive arm, a sample size of 1125 patients in each arm would be required to detect an absolute reduction of 5%, with 90% power, and a type I error of 5% (Table 45). If we were to include Infarction as a diagnosis only if TnI preceding anaemia was normal (7.4%), then a sample size of 387 in each arm would be required in each arm. If we were to increase the MCID required to 10%, this would reduce numbers to 241, and a reduction in power from 90% to 80% would reduce numbers further to 180 in each arm.

7.7.2. Primary outcome: 90 day mortality

Using data from TROPICCAL, rates of 90 day mortality that might be seen in a restrictive arm of a transfusion threshold trial would be approximately 29.0%. To detect an absolute reduction of 5%, with 90% power, and a type I error of 5%, a sample size of 1652 patients in each arm would be required.

7.7.3. Primary outcome: Composite Myocardial Infarction or death at 90 days.

In TROPICCAL the rate of 90 day mortality and/or Infarction was 47.5%. Using this as a single rate, then 2076 patients would be required to detect a MCID of 5%. It would be possible to develop the scale of the composite outcome further, as described above, to reduce these numbers.

Table 45: Potential sample sizes for trials for different primary outcomes, different minimal clinically important difference (MCID), and different power.

Primary Outcome	Rate	MCID	Type I error	Power	Sample size in each arm
Myocardial Infarction	7.4	5	5	90	387
	18.5	5	5	90	1125
	18.5	10	5	90	241
	18.5	10	5	80	180
90 day mortality	29.0	5	5	90	1652
Composite	47.5	5	5	90	2076

7.8. Conclusion

There is a high prevalence of patients with CVD in the UK ICU population, and a high proportion of these patients are exposed to significant anaemia. There is biological plausibility, and now evidence from our systematic review, that patients with co-existing CVD who are randomised to restrictive transfusion thresholds have an increased risk of new ACS. However, the exact population who might benefit from higher thresholds, and the optimal threshold for these patients remains uncertain, and a trial in this population is warranted.

I have presented a potential trial design for these patients, and have discussed concerns I believe are key to the design of an effective trial which would be most likely to find a difference if it exists.

8. Discussion

8.1. Summary of Findings

8.1.1. Systematic review of blood transfusion thresholds in patients with cardiovascular disease

- We found 11 trials enrolling 3,033 patients with CVD. There was no difference in 30 day mortality between patients randomised to restrictive and liberal transfusion thresholds (RR 1.15, 95% CI 0.88 to 1.50).
- There was a 78% increased risk of new Acute Coronary Syndrome in patients with co-existing cardiovascular disease who were randomised to a restrictive transfusion threshold compared to a more liberal threshold (RR 1.78, 95% CI 1.18 to 2.70).
- This suggests that a restrictive transfusion threshold may not be as safe as a more liberal transfusion threshold. However, there were different methods for reporting the diagnosis of ACS between studies, and the lack of blinding of clinicians/investigators to transfusion threshold allowed the possibility of ascertainment bias. A definitive, reproducible definition of Acute Coronary Syndrome is required, particularly in the ICU environment where patients are unable to communicate symptoms.

8.1.2. Early TnI and its association with hospital mortality (all general ICU patients)

- There was a significant association between TnI taken within 24 hours of ICU admission and hospital mortality (OR per doubling TnI 1.16, 95% CI 1.13, 1.20). This association persisted after adjustment for APACHE II model components, but was substantially attenuated (OR TnI 1.05, 95% CI 1.01, 1.09).
- TnI correlated most strongly with the Acute Physiological Score component. This represents acute physiological stress, and comprises surrogate markers of supply (hypoxia, hypotension, anaemia) versus demand (increased metabolic rate) imbalance, supporting the hypothesis that myocardial injury may be due to oxygen supply-demand imbalance.
- The addition of TnI did not improve the performance of the APACHE II risk prediction model, and we would not advocate the adoption of routine TnI analysis for general ICU patients on their admission to critical care.

8.1.3. TROPonin I in Cardiovascular patients in CriticAL care

- There was a high incidence of myocardial injury in critically ill patients with CVD. 71% of patients had a peak TnI greater than the sex-specific diagnostic threshold (“Injury”), and 24% of patients had a peak TnI greater than the sex-specific diagnostic threshold and dynamic changes on ECG consistent with ischaemia (“Infarction”). TnI consistently showed a rise-and-fall pattern consistent with an acute myocardial “hit” rather than persisting injury.
- 35.7% of patients had abnormalities associated with potential ischaemia on their admission ECG, and half of these patients had no subsequent dynamic changes. A third of patients with dynamic ECG changes consistent with ischaemia in the first five days of ICU had no signs of ischaemia on their ICU admission ECG. This highlights the importance of performing serial ECGs in this population. Only 4.4% of patients were diagnosed with Infarction by clinicians.

- Dynamic isolated T wave inversion had poor agreement between cardiologists blinded to clinical (and TnI) information, and poor discrimination for TnI elevation. We would advocate the exclusion of isolated T wave inversion in the diagnosis of Infarction in critically ill patients with CVD.
- Markers of ischaemia (lactate, ischaemic changes on ECG, anaemia) were more strongly associated with peak TnI than markers of inflammation (C-Reactive Protein), suggesting that in critically ill patients with co-existing CVD, myocardial injury may be at least in part due to oxygen supply-demand imbalance.
- Injury and Infarction were associated with worse mortality up to six months than No Injury. The magnitude of TnI had a stronger association with six month mortality for the Injury group, compared to the Infarction group. This suggests that if the patient had an ECG based ischaemic event this was the dominant issue, rather than the TnI value, whereas if the patient only had TnI elevation then the magnitude was more closely related to ultimate mortality. This supports the hypothesis that Infarction is a separate category from Injury. This has implications for subsequent investigation and management of these patients.
- Manipulation of physiological parameters such as anaemia, hypotension and tachycardia (as represented by severity of illness scores), that were associated with increased TnI may benefit patients at risk of myocardial injury.

8.1.4. Early TnI and its association with hospital mortality (ICU patients with co-existing CVD)

- In patients with co-existing CVD, there was a significant association between TnI taken within 24 hours of ICU admission and hospital mortality (OR per doubling TnI 1.20, 95% CI 1.07, 1.34). This association persisted after adjustment for APACHE II model components, but was substantially attenuated (OR TnI 1.15, 95% CI 1.01, 1.30). The point estimates for TnI were greater in this population with CVD compared with the general ICU population, however the confidence intervals were wide in the tropical dataset, reflecting its comparative size and overlapped the Glasgow dataset.
- The addition of potential ischaemia on the admission ECG did not improve the discrimination of the model. This may have been in part due to the high proportion of patients in TROPICCAL who had chronic non-dynamic abnormalities on their admission ECG, and the high proportion of patients who presented with a normal ECG but developed ischaemic changes in the following days.
- This highlights the limitations of restricting the dataset to the first day of ICU admission, with the use of admission instead of peak TnI, and potential ischaemia rather than confirmed dynamic ischaemia on ECG. This is important to bear in mind for the design of future trials, where early stratification may result in misclassification of patients.

8.1.5. TRAnsfusion requirements in Cardiovascular patients in Critical care (TRACC) protocol

- Patients randomised to a restrictive transfusion threshold in our Systematic Review had an increased risk of ACS, but there was no difference in 30 day mortality. In TROPICCAL, anaemia was independently associated with mortality at six months in critically ill patients with CVD. This suggests

that patients with co-existing CVD may benefit from higher oxygen delivery to the myocardium to reduce oxygen-supply demand imbalance. Myocardial Infarction is an appropriate primary end-point for this study.

- Thresholds of 70g/l (Restrictive) and 90g/l (Liberal) are within standard practice, and supported by the UK ICU community.
- 59.1% of patients in TROPICCAL would be eligible within seven days of ICU admission. 18.5% of these patients had a Myocardial Infarction that could be temporally attributable to exposure to significant anaemia (Hb≤90g/l) rise in TnI and ECG changes after their Hb fell below 90g/l, and 1125 patients in each arm would be required to detect an absolute reduction of 5%.

8.1.6. Recommendations

- A more liberal transfusion threshold of at least 80g/l in patients with co-existing CVD
- Systematic use of sequential ECGs in ICU to screen for myocardial injury in "at risk" patients
- Manipulation of physiological parameters such as anaemia, hypotension and tachycardia should be considered for patients with dynamic ECG changes plus troponin increase consistent with Infarction.

8.2. Implications for research and policy

Cardiovascular disease is common in the UK, and patients with co-existing CVD accounted for nearly a quarter of ICU admissions during the TROPICCAL study. The systematic appraisal of ECGs and daily TnI has identified a population at high risk of myocardial injury and infarction, which was not picked up by clinical investigation. Markers of ischaemia were independent predictors of troponin elevation. Patients who had myocardial injury or infarction during the first ten days after their ICU admission had high mortality rates up to six months. This supports the findings from our systematic review where patients randomised to a restrictive transfusion threshold had a greater risk of acute coronary syndrome.

There are significant implications for prevention and management of myocardial injury in critically ill patients with CVD. It is unknown whether appropriately identifying these patients as acute coronary syndrome and treating them as such will improve clinical outcomes. The vast majority of these patients were on secondary cardiac protection prior to ICU admission, but it remains unclear which patients continued their medication during their ICU stay. It may be beneficial for the higher risk patients, picked up with our systematic screening, to continue on cardiac protection such as aspirin, statins and beta-blockers, whereas it may be appropriate to pause it in patients at lower risk of myocardial injury.

In Chapter 7 I have outlined a proposal for a blood transfusion threshold trial with Myocardial Infarction as the primary end-point. This is appropriate given the increased risk of ACS in this population that we saw in our systematic review, and the independent association between anaemia and both TnI elevation we observed in TROPICCAL. This is an important question which will directly affect over ten percent of all patients admitted to ICU.

The observations in this thesis suggest that ischaemic supply demand imbalance is an important cause of TnI elevation in critically ill patients with CVD. However, this has been based on the use of ECGs and biomarkers, and we have been unable to visualise damage to the myocardium directly. I have designed two imaging studies that aim to differentiate between regional and generalised myocardial injury (8.2.1 and 8.2.2), and will enable us to target treatment more precisely to patients who are more likely to benefit.

Blood transfusion thresholds are a crude surrogate for estimating oxygen delivery, and future research should be aimed at individualised measurement of the oxygen supply-consumption balance in organs at high risk of ischaemia, such as the brain and myocardium.

There are other interventions that could be aimed at this high risk group to improve their long-term outcomes. These primarily could be focused at manipulating physiological parameters such as heart rate control or mean arterial pressure in order to maximise myocardial oxygen supply, and minimise myocardial oxygen demand. This thesis will inform these future interventions.

8.2.1. Future study: Strain transthoracic echocardiography:

Transthoracic echocardiography (TTE) is portable and has enabled imaging of critically ill patients, without the hazardous transfer of patients to CT or MRI scanners. TTE provides ultrasound images of the myocardium in real time, enabling assessment of cardiac size, structure, function and haemodynamics. It also allows for the identification of regional wall motion abnormalities (RWMA), produced by severe ischaemia that can be visualised within seconds of coronary artery occlusion by TTE. These changes occur prior to the onset of symptoms or changes on ECG. RWMAs reflect a localised decrease in the amplitude and rate of myocardial excursion, as well as a blunted degree of myocardial thickening and local remodelling (291). RWMAs are not specific to acute myocardial ischaemia, and may also be present in old infarction, focal myocarditis, prior surgery, left bundle branch block, ventricular pre-excitation via an accessory pathway, and cardiomyopathy. Furthermore, TTE may miss small but important RWMAs – an injury involving >20% of myocardial wall thickness may be required to detect a RWMA (234). However, critical illness in the form of vasodilation, mechanical ventilation and vasopressor/inotropic support all affect the interpretation of these scans (84, 292). Patients with septic shock frequently suffer from systolic and diastolic dysfunction (293). In addition to this, many patients cannot be moved into the optimal position for imaging, and lighting may be poor. Images are operator dependent, and inter-observer variability can be high (294). In critically ill patients in ICU 25-30% of TTE have incomplete endocardial resolution (295). This means that standard TTE may have relatively low sensitivity and specificity for small cardiac pathology in critically ill patients. Transthoracic echocardiography (TTE) is technically more difficult in these patients, and has only moderate diagnostic accuracy (296).

8.2.1.1. Strain echocardiography

Strain echo, is a relatively novel imaging technique and describes the lengthening, shortening, or thickening, also known as regional deformation of the myocardium (297). It is a post-processing computer algorithm that uses the unique "speckle" pattern visible in the myocardium on routine echo images. A user-defined region of interest is placed on the myocardial wall. The algorithm follows the movement of blocks of speckle pattern within this region over time frames, and is able to capture longitudinal, circumferential and radial strain (rate of deformation). The location shift of these markers from frame to frame provides the spatial and temporal data. Temporal alterations in these stable speckle patterns are identified as moving further apart or closer together, creating a series of regional strain vectors (297). This algorithm results in objective analyses of myocardial function, and is more sensitive than standard TTE evaluation of left ventricular function. Ischaemic RWMAs are often associated with passive motion, and strain is able to differentiate active contraction from passive motion (298), which is often difficult to differentiate visually. Strain has been validated in animal (298) and human (299) models of myocardial ischaemia secondary to acute coronary occlusion. The use of strain TTE enables greater diagnostic accuracy and inter-observer agreement compared with standard TTE (297, 300).

8.2.1.2. Strain echocardiography in critical care

There have been few studies using strain echocardiography in critical care. Two studies have performed strain echocardiography in adult patients with septic shock (300, 301). De Geer et al found that global longitudinal peak strain (GLPS) was frequently reduced in patients with septic shock (n=50). The impairment of GLPS correlated to systolic and diastolic left ventricular (LV) function, and to cardiac biomarkers, but remained unchanged over time, even after clinical recovery (300). Orde et al (n=60) found that strain echocardiography

detected more ventricular dysfunction (Right ventricle (RV) 72% vs 32% standard TTE; LV 69% vs 33% standard TTE). RV free wall longitudinal strain was associated with six month mortality (OR 1/10, 95% CI 1.02 to 1.26, p=0.02), but LV dysfunction was not associated with survival outcomes(301). Cinotti et al found that 37% of patients (n=46) with severe subarachnoid haemorrhage had GLS changes compatible with a stress cardiomyopathy whilst LV ejection fraction was preserved (302).

8.2.1.3. Study Aims and Objectives

Aim: Is it feasible to apply strain technology to TTE images in critically ill patients with cardiovascular disease?

Objectives:

- Does global longitudinal strain reflect other markers of myocardial dysfunction, primarily TnI concentration?
- Is the pattern of dysfunction different in patients classified as "Infarction" (dynamic TnI elevation above sex-specific diagnostic threshold with ischaemic changes on ECG), "Injury" (TnI elevation, but no ECG changes), and "No Injury" (no TnI elevation, no ECG changes) as per Chapter 4?
- Does strain have better diagnostic sensitivity and specificity compared to standard echo images?
- Do strain abnormalities persist into recovery, or resolve with the resolution of critical illness?

8.2.1.4. Proposal

We will recruit two cohorts of 20 patients in Edinburgh and Oxford. Each patient will have two echos (Edinburgh: pre elective TAAA surgery and day 3-5 post-operatively; Oxford: day 3-5 of ICU admission, and on the hospital ward in the recovery phase).

We will perform offline strain analysis of the 16 segment model of the left ventricle using speckle tracking (strain, strain rate and strain dysynchrony) looking for regional wall motion abnormalities (both systolic and diastolic). We will perform strain analysis of the left ventricle looking for a reduction in global longitudinal strain (GLS). We will use cut-off values of 15% for longitudinal strain, -21% for circumferential and -35% for radial strain as they are the lowest "normal" values. We will use the standard deviation of time-to-peak strain (mechanical dispersion) as this can give a strong indication of regional wall motion abnormalities. Images will be analysed using EchoPac (version 112, GE Ultrasount, Horten, Norway) in Edinburgh, and on the TomTec platform in Oxford.

8.2.2. Future Study: Cardiac Magnetic Resonance (CMR) and Computed Tomography Coronary Angiography (CTCA)

The observations in this thesis suggest that there is a modifiable ischaemic component causing myocardial oxygen supply-demand imbalance in critically ill patients with cardiovascular disease. It may be possible to target physiological parameters in order to reduce the incidence and/or impact of myocardial injury in these patients. However, the relative contribution of ischaemic versus inflammatory injury is unknown. It is important to understand the aetiology of TnI elevation as this has different therapeutic and prognostic implications. Current diagnosis is limited to ECG analysis and bedside transthoracic echocardiography. We have shown that ECG agreement is moderate for most abnormalities, even when assessed by cardiologists. We have also shown that transthoracic echocardiography is technically difficult in ICU patients, and the views obtained are frequently inadequate for objective methods of analysis such as strain.

Siddiqui et al performed Cardiac Magnetic Resonance (CMR) on seven non-cardiac patients with sepsis and detectable troponin I (using a high-sensitivity assay) whilst the patients were critically ill. They found no evidence of subendocardial infarct in any patients, but demonstrated increased myocardial oedema. This was predominantly in the epicardium indicating a non-ischaemic pattern of injury potentially secondary to myocardial inflammation or altered myocardial metabolism (303).

CMR: CMR can distinguish MI from other mechanisms of troponin release by distinguishing between subendocardial and other patterns of fibrosis (80). We will focus on the distribution of tissue oedema (T2) seen in acute myocardial injury (296, 304), enabling differentiation between regional ischaemic injury from non-ischaemic global inflammatory change, and the presence of late gadolinium enhancement that occurs in irreversible myocardial necrosis (296). T2 oedema persists for up to five weeks after myocardial infarction.

CTCA: CTCA can quantify coronary heart disease based on luminal diameter, atherosclerotic plaque and type of plaque, and assess cardiac and coronary structure, function, perfusion and viability. Patients with calcified atherosclerotic plaque are more likely to have non-calcified "soft" plaque that is prone to rupture and acute coronary thrombosis (305).The combination of CMR and CTCA will enable a detailed assessment of tissue injury patterns (CMR) and their association with atheromatous coronary artery stenosis (CTCA).

8.2.2.1. Proposal

Population: We will recruit two groups of ICU survivors with CVD. Group 1 (n=15): patients with "Infarction" (TnI elevation greater than sex specific diagnostic threshold, 34ng/l for men, 16ng/l for women and dynamic ECG changes); Group 2 (n=15): patients with "Injury" (TnI elevation greater than sex specific diagnostic threshold, 34ng/l for men, 16ng/l for women, but no ECG changes). T2 weighted oedema persists for up to five weeks after MI, so it is reasonable to wait until extubation as this will make it much safer to transfer patients to the MRI.

CTCA: Non-contrast ECG-triggered acquisition for calcium scoring and a post-contrast ECG-gated acquisition covering the whole of the heart and the root of the aorta. The maximum total research dose for the study is 10mSv. Iodine based contrast agent will be administered intravenously using standard local procedure.

CMR: Standard cardiac imaging breath-held ECG-gated sequences will be used to acquire 2-chamber, 4-chamber, long axis and short axis views of the heart. For each of the 4 time points, a breath-held inversion recovery sequence will acquire delayed enhancement images after a delay of 10-15 min following intravenous gadolinium contrast (0.2 mmol/kg). Cardiac oedema will be assessed by T2-weighted MRI with bright-blood (306). T2-weighted multi-echo gradient-echo sequences, breath-held and cardiac gated.

8.2.2.2. Data Analysis

MRI Analysis: Quantification of left ventricular mass, ejection fraction and late gadolinium enhancement infarct size will be determined using established protocols by independent blinded observers. Late enhancement analysis and T2 oedema volume will performed using quantification of infarct size by the full-width, half-maximum criterion. Optimisation of T2-weighted images will be done using a susceptibility gradient mapping post-processing technique.

CTCA: Volume and distribution of atheroma will be measured and quantified. Parametric variables will be compared using ANOVA (repeated measures) and unpaired/paired Student's t-test. Non-parametric variables will be compared using the Mann Whitney U/Krushkal-Wallis test, and categorical data using Fisher's exact test. Multivariable regression analysis will be performed to explore for associations. Statistical significance will be taken as two-sided $P<0.05$.

8.3. Conclusion

My work has shown an increased risk of ACS in patients with CVD randomised to restrictive transfusion thresholds. TnI elevation is prevalent in general ICU patients, and is independently associated with hospital mortality. A systematic approach to the detection of myocardial injury in critically ill patients with co-existing CVD who are unable to communicate symptoms, can identify a high risk population who have poorer survival than patients with no injury. Markers of ischaemia are more associated with TnI rise than markers of inflammation, supporting the hypothesis that myocardial injury in this population is at least in part due to oxygen supply-demand imbalance "myocardial infarction".

From this work, I would recommend (i) a more liberal transfusion threshold of at least 80g/l in patients with co-existing CVD; (ii) systematic use of sequential ECGs in ICU to screen for myocardial injury in 'at risk' patients; and (iii) manipulation of physiological parameters such as anaemia, hypotension and tachycardia should be considered for patients with dynamic ECG changes plus troponin increase consistent with Infarction. Future research should include 'precision medicine' trials in the substantial cohort of ICU patients with co-existing CVD to explore whether interventions that increase myocardial oxygen supply and/or treat infarction alter outcomes.

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